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**NITROSODIMETHYLAMINE -  
BIODEGRADATION**

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| <p>N-nitrosodimethylamine (NDMA) is biodegraded by microorganisms under certain conditions. In batch aqueous and soil studies the initial rates of mineralization increase with increasing concentration of NDMA, (ppt to ppm) however, the total percent or extent of mineralization decreases with increasing initial concentration. We were unable to demonstrate significant rates of biodegradation in continuous culture systems under anaerobic or aerobic conditions with a range of concentrations of NDMA. These limits on biodegradability indicate that a biological treatment mode, with the exception</p> |  |   |

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7 of a laboratory scale trickling filter containing activated charcoal, may not be a feasible alternative to deal with NDMA-laden wastewaters. The granular activated charcoal filter system demonstrated an ability to mineralize NDMA at feed concentrations of 50 ppm and 100 ppm. This system appears to warrant further investigation or scaleup for potential applicability for biological treatment of NDMA contaminated waters. Formaldehyde and methylamine were identified as transitory intermediates formed during the biodegradation of NDMA.

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## PREFACE

N-nitrosodimethylamine, a toxic and hazardous nitrosamine, was identified in the wastewater at Holston Army ammunition Plant. This wastewater originated from an ammonia recovery distillation column that received cyclonite (RDX) manufacturing wastewater. It was essential to understand the fate of this nitrosamine in the environment and to determine whether biological treatment is a feasible approach for the elimination of this hazard.

This work was performed for the US Army Toxic and Hazardous Materials Agency (USATHAMA) under project 1L161102AH68, W-61, P112.03.02, 33214137000. This represents a final report for this project. An interim report "Decomposition of N-nitrosodimethylamine in aqueous and soil Systems" technical report NATICK/TR-84/007, August 1983, was previously published.

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## N-NITROSODIMETHYLAMINE - BIODEGRADATION

### INTRODUCTION

An interim report previously published summarized our first year of study on the decomposition of N-nitrosodimethylamine in aqueous and soil systems.<sup>1</sup> The background information on the project and a literature review were included in that report as well as details of the experimental results from the first year's studies.

In this final report the results of our second year of study on the biodegradation of NDMA are detailed, including batch studies, continuation of the trickling filter columns, continuous flow systems, the effect of NDMA concentration on cell viability, resting-cell and cell-free studies, the identification of microbially derived metabolic intermediates, and studies on the kinetics of NDMA degradation.

A discussion on the implication of these findings for potential biological treatment of wastewaters containing NDMA is also presented.

### MATERIALS AND METHODS

#### Chemicals.

NDMA (N-(methyl-<sup>14</sup>C), lot 1337-123, 47 mCi per mmol, 99% pure) formaldehyde (<sup>14</sup>C-labeled, lot 1757-071, 10 mCi per mmol, 1% aqueous solution, approximately 95% pure), dimethylamine hydrochloride (N-(methyl-<sup>14</sup>C), lot 1625-093, 35.8 mCi per mmol, in methanol, 99% pure), and methylamine hydrochloride (N-(methyl-<sup>14</sup>C), lot 1397-208, 46 mCi per mmol, in ethanol, 99% pure) were purchased from New England Nuclear, Boston, MA. Unlabeled NDMA, 99% pure, was purchased from Aldrich Chemical Co., Milwaukee, WI.

#### High Performance Liquid Chromatography (HPLC).

The HPLC system used to quantitate NDMA was previously described.<sup>1</sup> To identify potential intermediates produced from NDMA, a radioactivity detector (FLO-ONE HS, Radiomatic Instruments Chemical Co., Tampa, FL) was coupled to the HPLC system. The use of <sup>14</sup>C-labeled NDMA resulted in the formation of <sup>14</sup>C-labeled inter-mediate detectable with this detector. Unlike NDMA, most of these compounds would otherwise not be detectable since they do not have significant UV absorbance. The correlation of retention times with those of the <sup>14</sup>C-labeled standards provided our initial indication of the identity of intermediates. The radioactivity detector was fitted with a 2.5-mL cell and received a total flow of 5.0 mL per minute (1.0 mL from the HPLC and 4.0 mL of Flo Scint III scintillation cocktail, Radiomatic). Peaks on the radioactivity chromatograms eluted approximately 1.3 minutes after the corresponding peaks on the HPLC ultraviolet detector in cases, such as with <sup>14</sup>C-NDMA, where the compound is detectable on both detectors. Background was in the range of 45 to 55 disintegrations per minute, and counting efficiency for the radioactivity detector was between 75% and 85%.

### Thin-layer Chromatography (TLC).

Hydrazine and substituted hydrazines (1,1-dimethylhydrazine and methylhydrazine) were detected on plastic backed cellulose plates without fluorescent indicator (Eastman Chemical, Rochester, NY). The developing solvent system was ethanol/water/hydrochloric acid (130/40/30). The hydrazines were visualized with a Folin-Ciocalteu reagent followed by exposure to ammonia fumes to develop the blue color. Samples from batch studies, with and without hydrochloric acid, were co-chromatographed along with the standards as hydrochloride salts. Acidified fractions were also concentrated 100-fold using a rotary evaporator at 60°C and then chromatographed.

To detect very low concentrations of the hydrazines, samples from batch studies initiated with  $^{14}\text{C}$ -NDMA were chromatographed along with the unlabeled hydrazine standard. The chromatograms of the unknowns, potentially containing  $^{14}\text{C}$ -labeled intermediates, were scraped off the plastic backing in twenty-two equal linear increments and counted for radioactivity in a Packard Tri Carb Model 3255 liquid scintillation counter. The  $R_f$  of the hydrazine standard was then correlated with the  $R_f$  of peaks of radioactivity.

### Gas Chromatography (GC) - purge and trap.

Many of the potential intermediates from NDMA are volatile (formaldehyde, methylamine, dimethylamine, methanol) and would only be formed in low ppb or ppt concentrations, considering a low initial concentration of NDMA is required for substantial total mineralization in batch studies. Therefore a concentration step followed by GC was one approach taken to identify these compounds. This procedure was carried out on samples from batch studies using a Hewlett Packard Model 7675A purge and trap sampler coupled to a Hewlett Packard Model 5840 GC.

A mixed standard containing 5  $\mu\text{L}$  each of formaldehyde, dimethylamine, methylamine, and methanol was made up in 50 mL of distilled deionized water that had been boiled for one hour. This standard was used to establish optimum purge and trap parameters for extraction of these potential intermediates. The optimum desorb temperature from the column was found to be 100°C for six minutes. A 4% Carbowax 20M and 0.8% KOH on Carbopack B packing in a 3-m-long by 0.32-cm-diameter glass column was used for separation of the compounds in the GC. A 12-minute purge time was found to be optimal, corresponding to a purge volume of 600 mL of nitrogen gas. The prepurge time was 5 minutes, the auxiliary temperature was 200°C, the injector temperature was 170°C, and the oven temperature was initially 65°C and increased at 15°C per minute up to 150°C.

### Mass Spectrometry (MS).

MS was performed on a SCIEX TAGA 6000 triple stage quadrupole mass spectrometer (SCIEX, Thornhill Ontario, Canada). Analysis of NDMA batch cultures was accomplished directly from filtered (0.45  $\mu\text{m}$  membrane filters) aqueous samples. Standards (NDMA; methylamine; dimethylamine; and

formaldehyde), were also analyzed. This equipment is well suited to the analyses of very low concentrations of volatile compounds in aqueous solutions. The results from these analyses provided confirmation for preliminary identifications of intermediates determined by HPLC-radioactivity.

#### Continuous Systems.

Two continuous flow systems (aerobic, anaerobic) were run for almost a year to evaluate the degradation of NDMA at low concentrations. The previous report had described similar results but for the most part for higher concentrations of NDMA.<sup>1</sup> For these new studies the changes in NDMA concentrations and media composition in the feed for both systems as well as percent change in NDMA concentration, the retention times, and the pH of the systems are described in the results section.

#### Trickling Filters.

Laboratory scale trickling filters with granular activated charcoal as the support medium were studied for over 500 days. The experimental details of the setup of the active and sterile (1% mercuric chloride) columns, including column sizes, support medium, and nutrient feed, were described previously.<sup>6</sup> We previously presented results covering the first year of operation and this report includes the follow-on data. The influent initially contained 100 ppm NDMA, which was reduced to 50 ppm for the majority of the experiment.

After the first 300 days of study a question remained as to whether the NDMA on the active column was being mineralized or simply sorbed. A higher sorption capacity of the active column had to be considered because the mercuric chloride may have inhibited the sorption capacity of the sterile column. In order to conclusively answer this question, both the active and sterile columns received one-time doses of  $^{14}\text{C}$ -NDMA, 2.36  $\mu\text{Ci}$  each. During the next 203 days the total radioactivity recovered in column effluents as well as in sodium hydroxide traps, 10 mL of a 1 N solution, was determined. Complete recovery of  $^{14}\text{CO}_2$  in the alkaline traps from the effluent gases was not possible with the system design already in place. However, despite this limitation, sufficient recovery was found to provide a qualitative evaluation of the sterile and active systems.

Samples of the microbial slime growing on the active charcoal column were removed and attempts were made to isolate active NDMA-degrading colonies. Standard microbiological techniques were used to isolate pure cultures followed by incubation with  $^{14}\text{C}$ -NDMA. Base traps were used to trap  $^{14}\text{CO}_2$  as a measure of activity. Incubations were either directly on agar petri plates or in liquid batch culture. Growth media included the same influent medium used for the trickling filters but with 1.5% granulated agar (Difco, Detroit, MI) and with or without glucose, 1 ppb, and nutrient agar (Difco). The NDMA, usually 100 ppm, was added in agar overlay medium. Growth was usually very sparse on the trickling filter media. All isolates were replated to assure viability on the defined media on which they were originally grown.

### Batch Studies.

A series of batch studies with  $^{14}\text{C}$ -NDMA, not reported in the interim report,<sup>1</sup> were initiated to further evaluate the kinetics of mineralization under a variety of incubation conditions. The experimental setup, trapping systems, incubation conditions, and inoculum were previously described. The details of the individual studies will be presented in the results section. All samples were corrected for quench with an external standard, and most samples were counted for 20 minutes. Batch and continuous cultures were inoculated with organisms from activated sludge (Marlborough Easterly Sewage Treatment Plant, Marlborough, MA), anaerobic sludge digest (Nut Island Sewage Treatment Plant, Boston, MA) and garden soil. One mL samples of the two sludges were combined with 1 gram of the soil, diluted with 50 mL of lake water, mixed, gravity filtered through filter paper, and 100  $\mu\text{L}$  used for inoculum in batch cultures and 1 mL in continuous cultures. This inoculum contained between  $2.3 \times 10^3$  and  $3.5 \times 10^3$  CFU/mL.

### Cell Viability - NDMA Concentrations.

Conditions were established to evaluate the effect of NDMA on cell viability. Concentrations of NDMA of zero (control), 1 ppb, 1 ppm, and 1000 ppm in filtered lake water with natural (field) populations of microorganisms were incubated for 24 hours. Samples, 100-mL, were withdrawn initially, at one hour and at 24 hours, run in a dilution series, and then spread on both nutrient agar (Difco) and filtered lake water supplemented with 1 ppb glucose and 1.5% granulated agar (Difco). Each plate was replicated twice. The two types of growth media were used to promote the growth of different segments of the natural population and thereby allow us to look for a change in one or both of these populations in response to exposure to NDMA. These two populations have been referred to as eutrophic and oligotrophic and growth would be encouraged by the nutrient agar and supplemented lake water agar, respectively.

### Soil Binding.

NDMA appeared to degrade more readily at slightly higher concentrations in soil than in water. Therefore, a study was undertaken to determine if NDMA binds to soil, which in turn could enhance its susceptibility to catabolic enzymes in the soil and thus its rate of degradation. Details of the technique used to study these types of binding reactions were previously described<sup>2</sup>. Humic acid (2000 ppm and 1000 ppm), the soil organic matter used in these studies, was reacted with 50 ppb NDMA in phosphate buffer at pH 7.0. Samples of the reaction mixture and controls consisting of buffered NDMA were withdrawn periodically, and the NDMA was quantified by HPLC.

### Resting Cell and Cell Extract Studies.

Cell extract studies were conducted to determine if the cell wall and cell wall transport mechanisms were responsible for the limitation of total mineralization of NDMA that was observed at the higher concentrations in the

batch studies. The resting cell studies permitted us to study the effect of increased biomass on the rates of mineralization of NDMA, and thus also provide us with a clearer picture of the interactions of concentration and mineralization.

Adapted (100 ppb NDMA) and unadapted cells were grown up overnight in nutrient broth, 4 g per liter (Difco), in a 37°C water bath. Cells were harvested by centrifugation and split into two fractions. One fraction was used for the adapted and unadapted whole cells, while the other cell fraction was treated by sonication (Branson Sonifier) to break up whole cells. Cell debris was removed by centrifugation. Studies were conducted in phosphate buffer, pH 7.0, with 10 ppm or 100 ppb concentrations of NDMA, the whole cells or the cell extract with both adapted and unadapted conditions, and a control series consisting of autoclaved setups. Systems were seeded with  $^{14}\text{C}$ -NDMA, 0.044  $\mu\text{Ci}$  in most cases, and the same acid and base trapping systems were used as those described for the batch studies. In some instances toluene was added to the incubations to inhibit whole cell activity.

#### Sorption on Charcoal.

Granular activated coconut charcoal (6-14 mesh, Fisher Scientific, Boston, MA) was evaluated with NDMA to determine sorption capacity. Solutions of NDMA, 5 ppm, 50 ppm, 500 ppm, 5000 ppm, and 10,000 ppm (each in 1 liter of distilled deionized water) were tested with and without (controls) 10 grams of charcoal. Samples from these solutions were withdrawn periodically, filtered through a 0.45- $\mu\text{m}$  membrane filter, and analyzed by HPLC.

### RESULTS

#### Continuous Systems.

The disappearance of NDMA at low concentration from both aerobic and anaerobic continuous flow systems is illustrated in Figures 1 and 2. The nutrient conditions, pH, retention times, and NDMA concentrations corresponding to these figures are described in Tables 1 and 2. In general, there is little indication of significant disappearance of NDMA from either system, despite influent concentrations between 100 ppb and 200 ppb for most of the experiment. There is some loss of NDMA, less than 10% throughout most of the study, and there are a few higher spikes indicating increased disappearance; 40% to 50%. However, there is no consistent pattern to indicate that the organisms in these systems were developing or maintaining an ability to degrade NDMA.

#### Trickling Filters.

Figure 3 illustrates the results of the charcoal column study run over 500 days. After reaching adsorption equilibrium on the columns, NDMA passes through the sterile column unchanged. Almost all of the NDMA fed onto the active column was removed, either through mineralization, adsorption or volatilization. The addition of  $^{14}\text{C}$ -NDMA to the columns provided the conclusive evidence that NDMA was actually being mineralized on the active column, Figure 4. After 203 days, 95.9% of the original  $^{14}\text{C}$ -NDMA added to the sterile column has been recovered in

the effluent, while only 2.7% of the total was recovered in the effluents from the active column. During the same time frame 6.5% of the total added  $^{14}\text{C}$ -NDMA was recovered as  $^{14}\text{CO}_2$  in the alkaline traps compared with 0.2% from the sterile column. These results clearly indicate that NDMA is being mineralized on the active charcoal column.

Figure 5 illustrates results obtained from efforts to isolate the NDMA-degrading microorganisms from the active charcoal column. During twenty days of incubation on solid media, the isolated colonies as well the mixed population produced  $^{14}\text{CO}_2$  from  $^{14}\text{C}$ -NDMA. The results show that the various isolates have different capabilities for mineralizing NDMA. Some of the isolates were also incubated in aqueous batch media. Table 3 presents results from the incubation of the mixed population isolated from the charcoal column.

TABLE 3. Total  $^{14}\text{C}$ -labeled Volatile Products Recovered during 27 Days.<sup>a</sup>

| <u>Incubation conditions</u>               | <u>Concentration<br/>NDMA (ppm)</u> | <u>Sterile (-)<br/>Active (+)</u> | <u>Base traps<sup>b</sup><br/>(% of total)</u> |
|--|-------------------------------------|-----------------------------------|--|
| Mycological agar with streptomycin         | 100                                 | --                                | 0.3  |
| Mycological agar with streptomycin         | 100                                 | +                                 | 1.0  |
| Fungal salts with glucose and streptomycin | 100                                 | --                                | 0.6  |
| Fungal salts with glucose and streptomycin | 100                                 | +                                 | 0.8  |
| Lake water with basal salts                | 100                                 | --                                | 0.5  |
| Lake water with basal salts                | 100                                 | +                                 | 1.0  |

<sup>a</sup>0.36  $\mu\text{Ci}$  per flask in 400 mL.

<sup>b</sup>changed four times the first week, twice a week for two weeks, and once a week there after.

A similar experiment was run as that just described, but the incubation conditions were modified to include charcoal in the batch media in an attempt to duplicate ingredients in the charcoal columns. Table 4 presents the results from this study.

The activity found in both of these batch aqueous studies was not comparable to the high rates of degradation found with the trickling filter columns. Despite using isolates from the column, in an effort to encourage the same activity that was found under the column conditions, the incubation conditions could not be duplicated in batch aqueous studies. Furthermore, it was noted that the addition of charcoal (the same charcoal as that used in the trickling filter study) almost completely inhibited all activity (Table 4). This same phenomenon was found in our previous studies.

TABLE 1. Aerobic Degradation of NDMA in a Continuous Flow System.

| Days    | (NDMA)<br>influent | Retention time<br>days | pH      | Media                                 | % NDMA<br>disappearance |
|---------|--------------------|------------------------|---------|---------------------------------------|-------------------------|
| 0-12    | 45.3±0.4 ppm       | 6.1±1.8                | 8.0±0.2 | Basal salts <sup>a</sup>              | 35.0±18.4               |
| 13-65   | 5.4±0.7 ppm        | 4.6±0.6                | 7.6±0.1 | Basal salts <sup>a</sup>              | 6.8±4.2                 |
| 66-127  | 5.1±0.4 ppm        | 4.4±0.4                | 7.5±0.2 | Basal salts <sup>a</sup>              | 9.0±0.0                 |
| 128-293 | 140.3±55.7 ppb     | 4.3±0.4                | 7.3±0.5 | Basal salts <sup>a</sup>              | 13.0±15.8               |
| 294-323 | 128.4±34.9 ppb     | 4.9±0.4                | 7.1±0.1 | Basal salts <sup>b</sup> ;<br>glucose | 40.2±22.9               |

<sup>a</sup>MgSO<sub>4</sub> · 7H<sub>2</sub>O, 500 mg/L; NaCl, 50 mg/L; CaCl<sub>2</sub>, 15 mg/L; FeCl<sub>3</sub> · 6H<sub>2</sub>O, 10 mg/L; CuSO<sub>4</sub> · 5H<sub>2</sub>O, 10 mg/L; MnSO<sub>4</sub> · H<sub>2</sub>O, 10 mg/L; NaMoO<sub>4</sub> · 2H<sub>2</sub>O, 1 mg/L; in Lake Water

<sup>b</sup>Basal salts; glucose, 1 g/L in Lake Water



TABLE 2. Anaerobic Degradation of NDMA in a Continuous Flow System.

| Days    | (NDMA)<br>influent | Retention time<br>days | pH      | Media                                 | % NDMA<br>disappearance |
|---------|--------------------|------------------------|---------|---------------------------------------|-------------------------|
| 0-65    | 45.6±1.1 ppm       | 4.7±1.0                | 8.3±0.1 | Basal salts <sup>a</sup>              | 1.8±1.3                 |
| 66-106  | 4.8±0.4 ppm        | 4.0±0.8                | 7.3±0.2 | Basal salts <sup>a</sup>              | 1.3±2.4                 |
| 107-181 | 5.3±0.6 ppm        | 4.7±1.2                | 7.3±0.4 | Basal salts <sup>a</sup>              | 5.1±5.4                 |
| 182-343 | 146.4±76.8 ppb     | 4.3±1.3                | 6.9±0.6 | Basal salts <sup>a</sup>              | 0.7±1.7                 |
| 344-378 | 149.3±15.5 ppb     | 4.3±0.5                | 7.0±0.1 | Basal salts <sup>b</sup> ;<br>glucose | 23.2±22.8               |

<sup>a</sup>MgSO<sub>4</sub>·7H<sub>2</sub>O, 500 mg/L; NaCl, 50 mg/L; CaCl<sub>2</sub>, 15 mg/L; FeCl<sub>3</sub>·6H<sub>2</sub>O, 10 mg/L; CuSO<sub>4</sub>·5H<sub>2</sub>O, 10 mg/L; MnSO<sub>4</sub>·H<sub>2</sub>O, 10 mg/L; NaMoO<sub>4</sub>·2H<sub>2</sub>O, 1 mg/L; in Lake Water

<sup>b</sup>Basal salts; glucose, 1 g/L in Lake Water

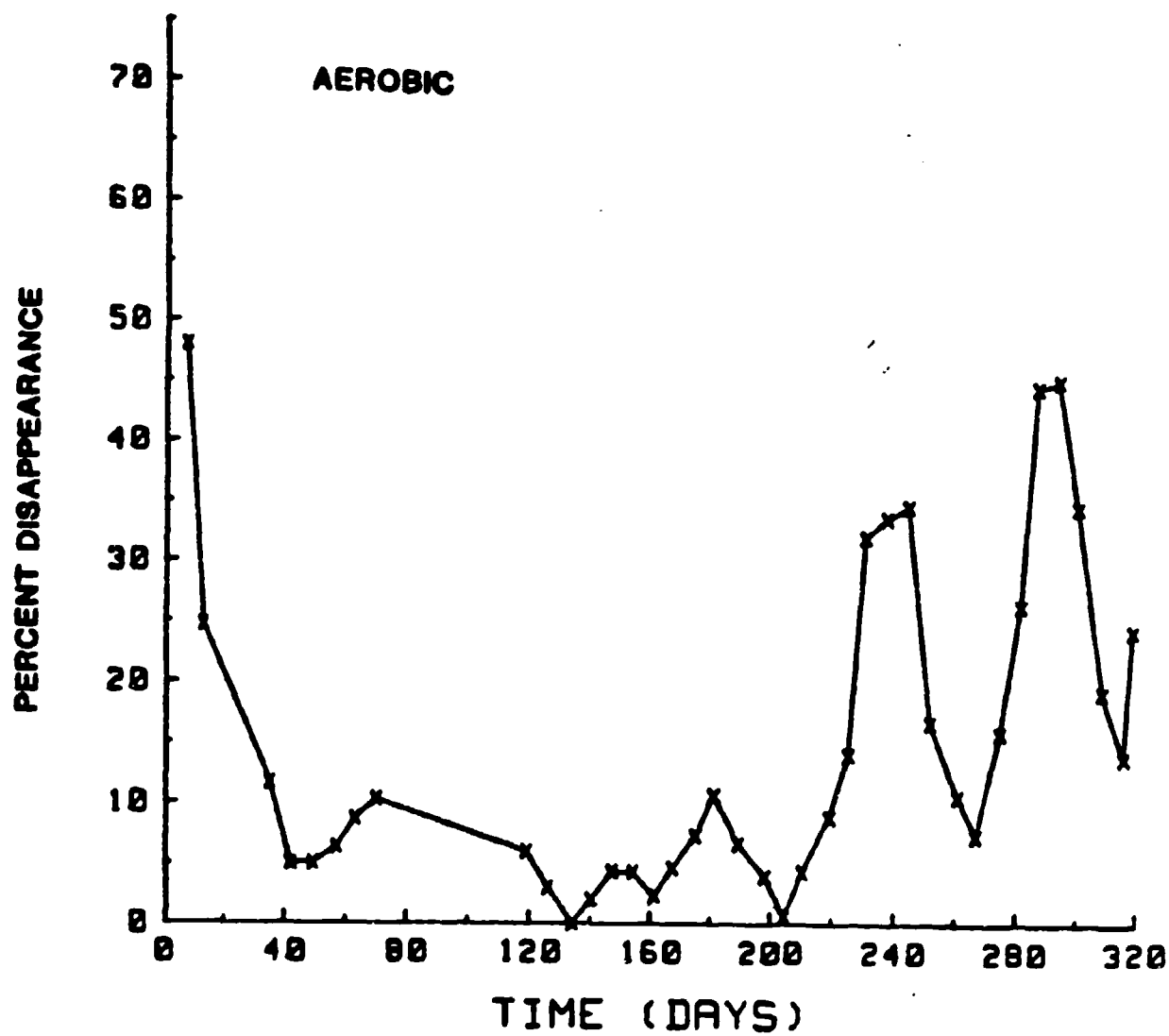


Figure 1. Disappearance of NDMA in continuous culture under aerobic conditions.

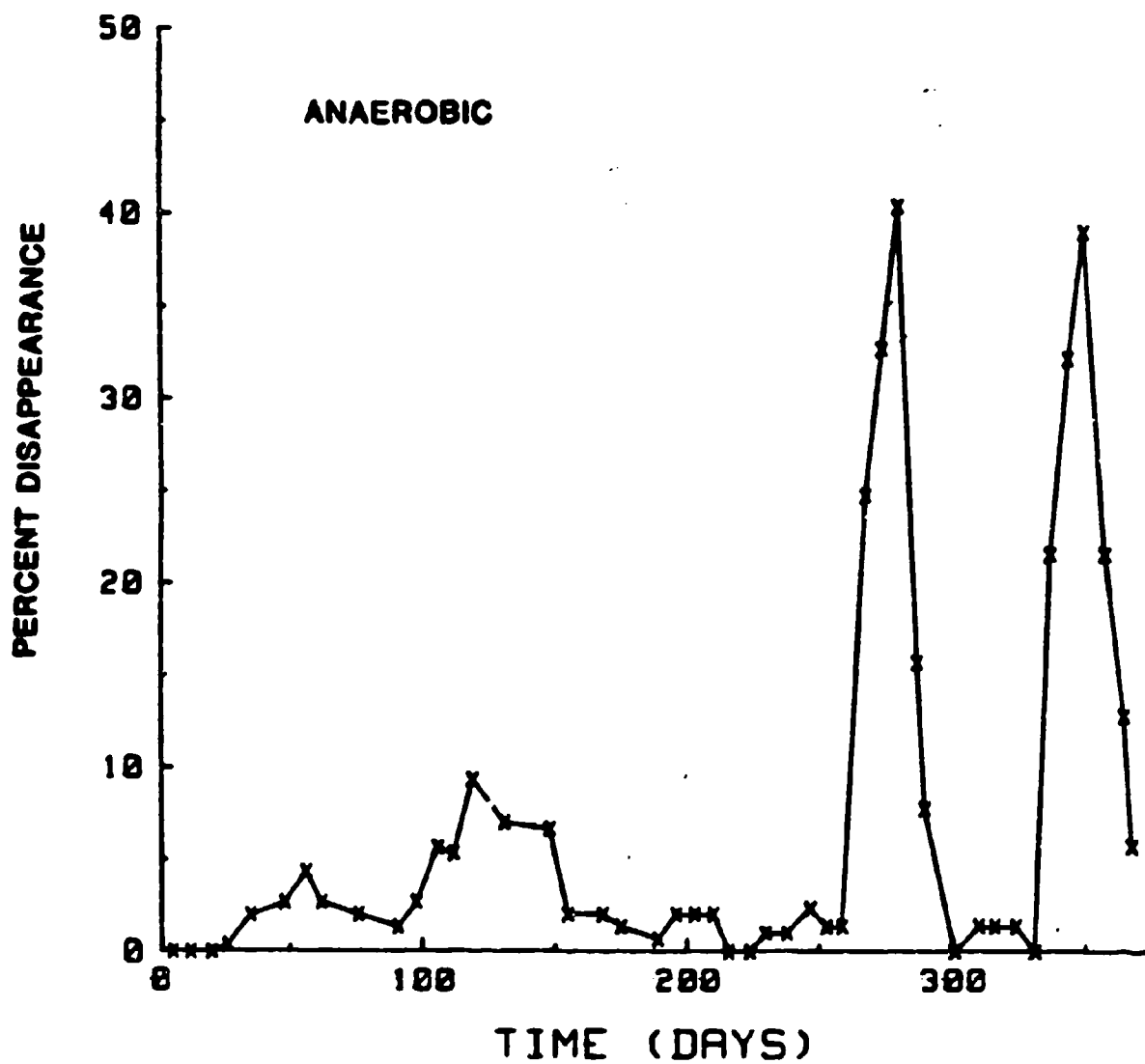


Figure 2. Disappearance of NDMA in continuous culture under anaerobic conditions.

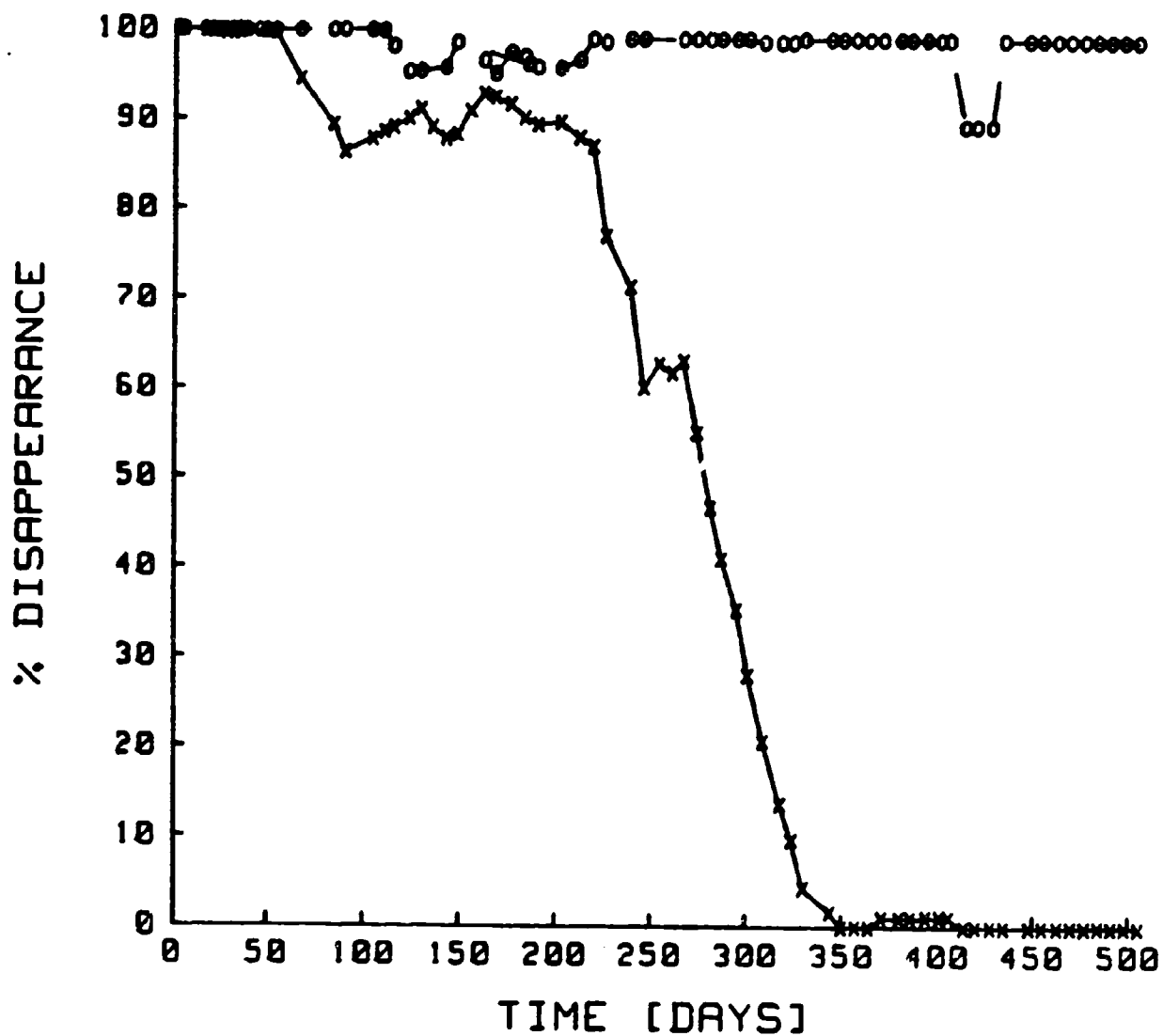


Figure 3. Disappearance of NDMA in laboratory scale trickling filters containing charcoal, active column (circles), sterile column (crosses).

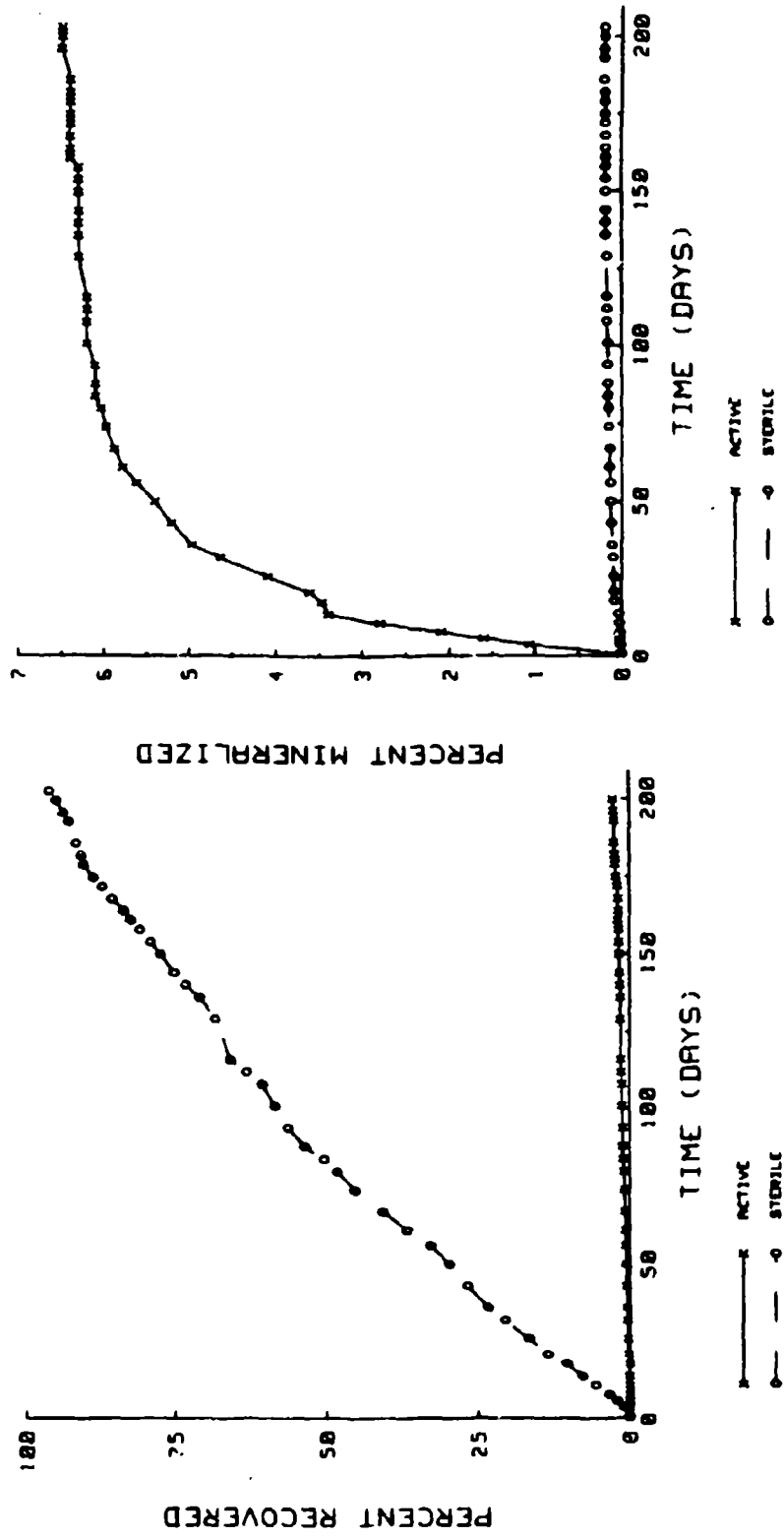


Figure 4. Recoveries of radioactivity from charcoal trickling filters in column effluents (left) and in base traps (right).

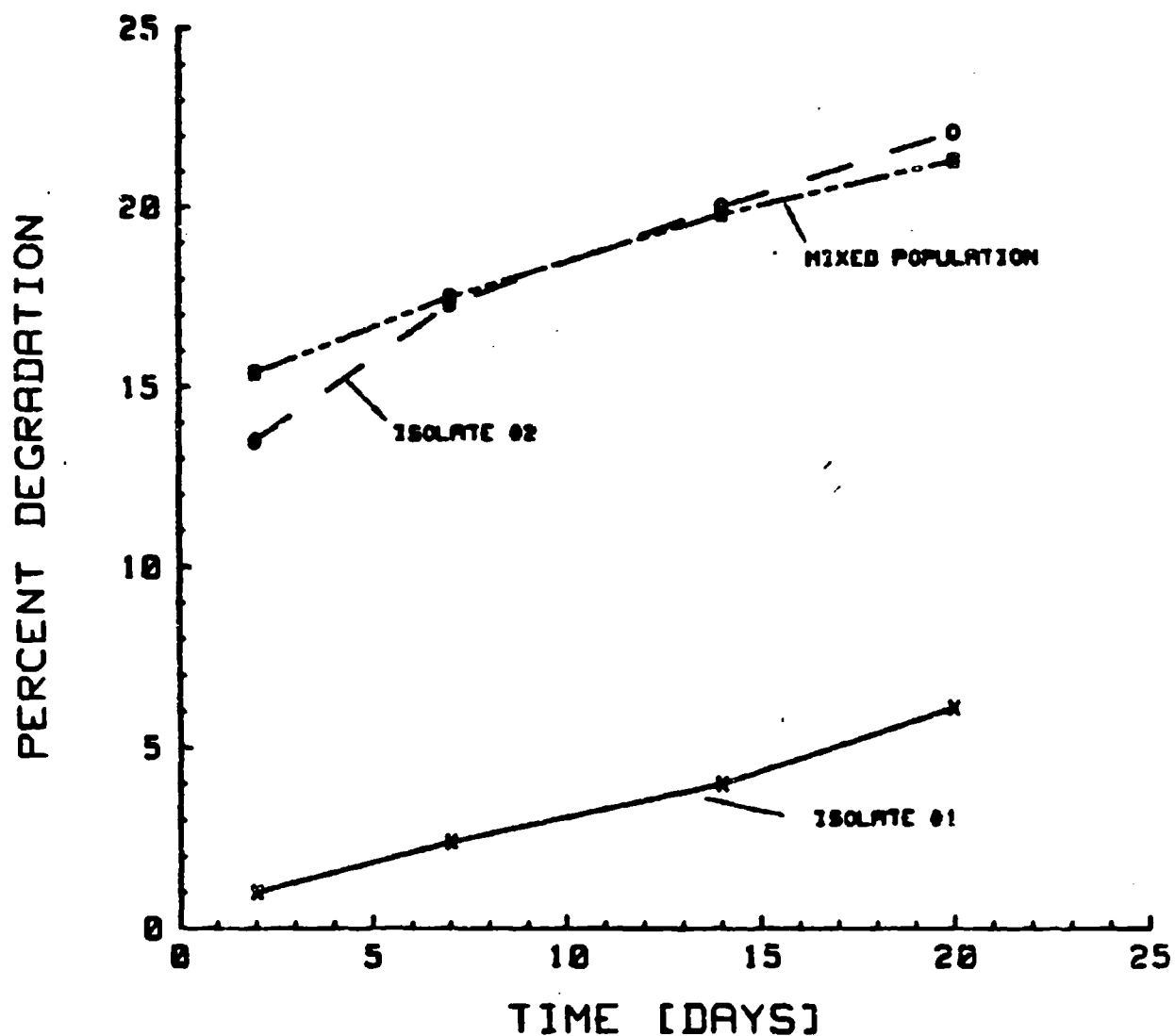


Figure 5. Recoveries of radioactivity from incubations of isolates from the active charcoal trickling filter column, 0.42  $\mu$ Ci NDMA per plate. Medium consisted of the trickling filter medium without glucose, granulated agar, and 100 ppm NDMA in an overlay. Sterile controls containing 1% mercuric chloride were run concurrently.

TABLE 4. Total  $^{14}\text{C}$ -labeled Volatile Products Recovered in Trickling Filter Isolate Study during 49 Days.<sup>a</sup>

| <u>Incubation conditions</u>                                | <u>Sterile (-)<br/>Active (+)</u> | <u>Base traps<sup>b</sup><br/>(% of total)</u> | <u>Acid traps<sup>b</sup><br/>(% of total)</u> |
|---|-----------------------------------|--|--|
| Lake water with salts                                       | +                                 | 15.3   | 0.2  |
| Lake water with salts and charcoal<br>(17.5 g) <sup>c</sup> | +                                 | 0  | 0  |
| Lake water with salts and charcoal<br>(35 g) <sup>d</sup>   | +                                 | 0.1  | 0  |
| Lake water with salts and glucose                           | +                                 | 16.2   | 0.3  |
| Lake water with salts, glucose, and<br>charcoal (17.5 g)    | +                                 | 0  | 0  |
| Lake water with salts, glucose, and<br>charcoal (35 g)      | +                                 | 0  | 0  |
| Lake water  | --                                | 0.7  | 0.2  |
| Lake water with charcoal (17.5 g)                           | --                                | 0  | 0  |
| Lake water with charcoal (35 g)                             | --                                | 0  | 0  |

<sup>a</sup>100 ppm NDMA and 0.157  $\mu\text{Ci}$  NDMA per flask in 50 mL

<sup>b</sup>changed three times the first week and twice a week thereafter.

<sup>c</sup>17.5 g of charcoal are completely submerged in the medium.

<sup>d</sup>35 g of charcoal are only partially submerged.

#### Batch Studies.

Table 5 and Figure 6 summarize the results of a batch aqueous study with  $^{14}\text{C}$ -NDMA to examine the influence of nutrients on rates of mineralization of low concentrations of NDMA (10 ppb) was examined. The results of this study further support our previous findings that maximum rates of mineralization are achieved in media which did not provide significant amounts of alternate organic carbon. The minimal medium lake water, supplemented with salts, provided the most suitable conditions for the degradation of NDMA, and this was the case with or without sodium sulfide present as a reducing agent. When this medium was supplemented with carbon as glucose or nutrient broth, the total mineralization of NDMA was dramatically reduced.

TABLE 5. Total  $^{14}\text{C}$ -labeled Volatile Products Recovered in SR Study during 90 Days.<sup>a</sup>

| <u>Incubation conditions</u>                          | <u>Sterile (-)<br/>Active (+)</u> | <u>Base traps<sup>b</sup><br/>(% of total)</u> | <u>Acid traps<sup>b</sup><br/>(% of total)</u> | <u>Residual<sup>c</sup><br/>radioactivity<br/>(% of total)</u> |
|---|-----------------------------------|--|--|--|
| Lake water with salts                                 | -                                 | 0.9  | 0.3  | 79.3   |
| Lake water with glucose                               | -                                 | 0.7  | 0.2  | 82.1   |
| Lake water with nutrient broth                        | -                                 | 0.7  | 0.3  | 71.9   |
| Lake water with salts and reducing agent <sup>d</sup> | -                                 | 0.5  | 0.3  | 78.0   |
| Lake water with glucose and reducing agent            | -                                 | 0.4  | 0.3  | 77.2   |
| Lake water with nutrient broth and reducing agent     | -                                 | 0.3  | 0.3  | 74.8   |
| Lake water with salts                                 | +                                 | 64.1   | 0.4  | 14.3   |
| Lake water with glucose                               | +                                 | 10.6   | 0.2  | 59.3   |
| Lake water with nutrient broth                        | +                                 | 13.6   | 0.3  | 53.3   |
| Lake water with salts and reducing agent              | +                                 | 60.1   | 0.4  | 4.1  |
| Lake water with glucose and reducing agent            | +                                 | 13.6   | 0.4  | 60.5   |
| Lake water with nutrient broth and reducing agent     | +                                 | 10.8   | 0.3  | 62.8   |

<sup>a</sup>10 ppb NDMA and 0.077  $\mu\text{Ci}$  NDMA per flask in 50 mL.

<sup>b</sup>changed twice a week for three weeks, once a week for seven weeks and once the last two weeks.

<sup>c</sup>at the completion of the study, aliquots of the media were assayed for residual radioactivity.

<sup>d</sup>0.025% sodium sulfide.



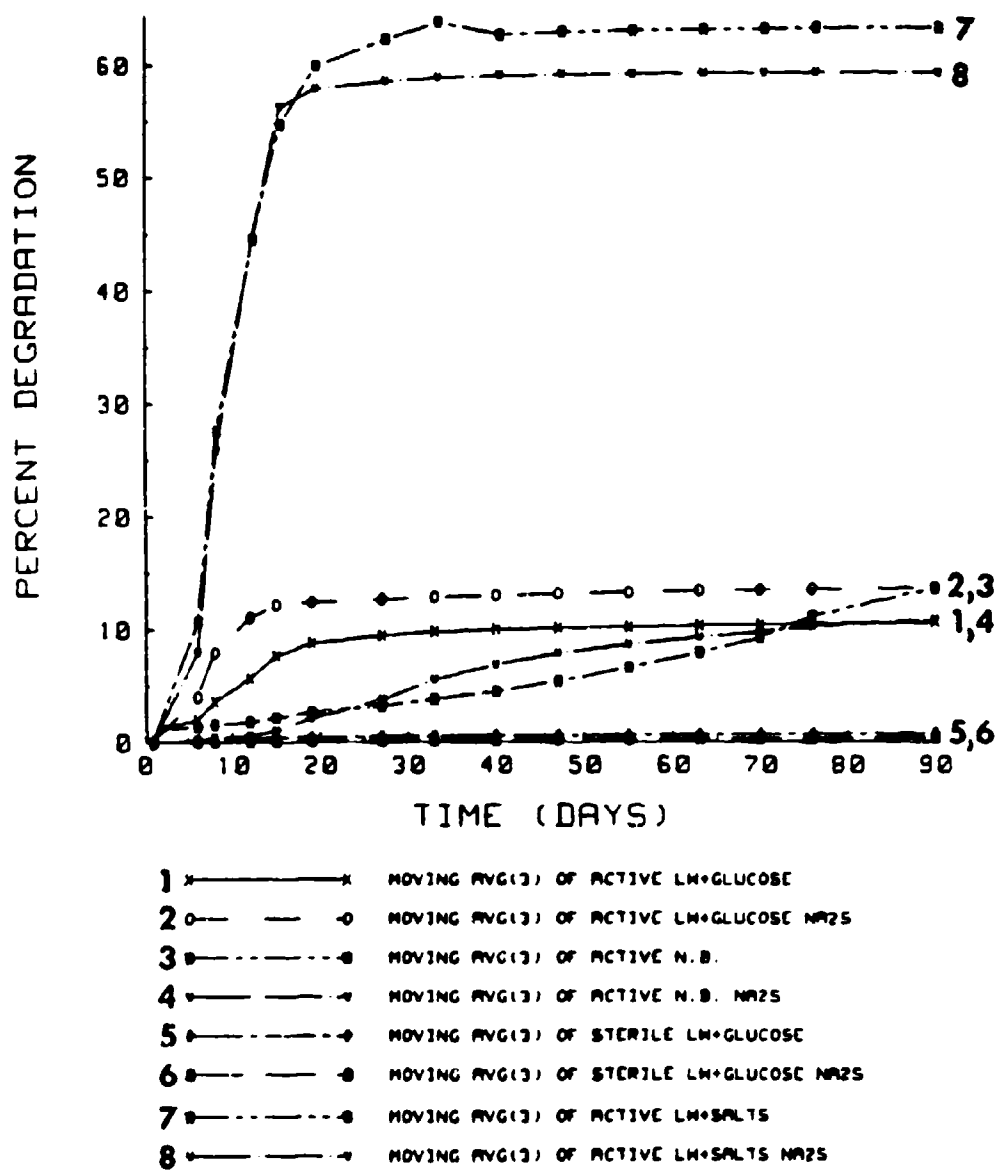


Figure 6. Mineralization of NDMA, 10 ppb, from lake water supplemented with various carbon sources and a reducing agent.

The residual radioactivity found in the flasks after the 90 days of incubation mirrors the mineralization results. In all cases there is about a 17% to 35% discrepancy in the total counts recovered after adding the counts trapped and the residual radioactivity counts in the media. The difference is most likely accounted for by the production of volatile gases not recovered by the trapping system, such as methane, as well as volatilization of the NDMA from the systems. These findings also agree with our earlier results from the continuous culture studies where at least 5% to 20% of the influent NDMA was lost from the systems. In the sterile flasks in this batch study the radioactivity not accounted for approximates 17% to 28%; a result fairly close in agreement with the continuous flow study results. In the active systems the recoveries are a little less than with the sterile systems, the difference most likely accounted for by the production of methane or other volatile products not retained in the acid and base trapping systems.

Table 6 presents results of a batch aqueous study with 1 ppm NDMA. The results are similar to previous findings in that at this concentration of NDMA only low rates of mineralization are found. As before, the addition of a nutrient supplement, glucose, inhibited some of the activity. The total recoveries of radioactivity followed the same pattern as before.

TABLE 6. Total  $^{14}\text{C}$ -labeled Volatile Products Recovered in the BM Study during 77 Days.<sup>a</sup>

| <u>Incubation conditions</u>                   | <u>Sterile (-)<br/>Active (+)</u> | <u>Base traps<sup>b</sup><br/>(% of total)</u> | <u>Acid traps<sup>b</sup><br/>(% of total)</u> | <u>Residual<sup>c</sup><br/>radioactivity<br/>(% of total)</u> |
|--|-----------------------------------|--|--|--|
| Lake water with salts                          | --                                | 0.4  | 0.1  | 82.0   |
| Lake water with salts and glucose <sup>d</sup> | --                                | 0.6  | 0.1  | 83.3   |
| Lake water with salts                          | +                                 | 16.8   | 0.1  | 56.6   |
| Lake water with salts and glucose              | +                                 | 10.8   | 0.1  | 63.3   |

<sup>a</sup>1 ppm NDMA and 0.667  $\mu\text{Ci}$  NDMA per flask in 200 mL

<sup>b</sup>changed twice a week for five weeks, once a week for four weeks, and once the last two weeks.

<sup>c</sup>at the completion of the study, aliquots of the media were assayed for residual radioactivity.

<sup>d</sup>1 g per liter glucose.

The influence of supplementation with glucose in aqueous media on rates of mineralization of 10 ppb NDMA is presented in Table 7 and Figure 7. The results again demonstrate the inhibitory action of supplemental carbon on total

mineralization of NDMA. The highest rate of mineralization was in lake water with salts without glucose, and rates dropped progressively in relation to the increasing concentrations of glucose. At most, about 70% of the total counts were recovered in the base traps as  $^{14}\text{CO}_2$ . The total counts recovered, when the trapped and residual radioactivity were combined, showed the same pattern as already described in regard to volatilization and untrapped gas production.

TABLE 7. Total  $^{14}\text{C}$ -labeled Volatile Products Recovered in the GS Study during 90 Days.<sup>a</sup>

| Incubation conditions <sup>b</sup> | Sterile (-)<br>Active (+) | Base traps <sup>c</sup><br>(% of total) | Acid traps <sup>c</sup><br>(% of total) | Residual <sup>d</sup><br>radioactivity<br>(% of total) |
|------------------------------------|---------------------------|---|---|--|
| glucose, 0                         | -                         | 0.5 <sup>e</sup>                        | 0.1 <sup>e</sup>                        | — <sup>f</sup>   |
| glucose, 0.1 g                     | -                         | 0.8                                     | 0.2                                     | 80.3   |
| glucose, 0.5 g                     | -                         | 0.8                                     | 0.3                                     | 79.3   |
| glucose, 1.0 g                     | -                         | 0.8                                     | 0.3                                     | 79.7   |
| glucose, 5.0 g                     | -                         | 0.7                                     | 0.3                                     | 79.3   |
| glucose, 50.0 g                    | -                         | 0.7                                     | 0.3                                     | 76.5   |
| glucose, 0 g                       | +                         | 67.1                                    | 0.3                                     | 5.2  |
| glucose, 0.1 g                     | +                         | 70.2                                    | 0.4                                     | 5.8  |
| glucose, 0.5 g                     | +                         | 40.1                                    | 0.4                                     | 33.2   |
| glucose, 1.0 g                     | +                         | 30.4                                    | 0.3                                     | 44.9   |
| glucose, 5.0 g                     | +                         | 18.2                                    | 0.4                                     | 68.4   |
| glucose, 50.0 g                    | +                         | 14.7                                    | 0.3                                     | 68.0   |

<sup>a</sup>10 ppb NDMA, 0.078  $\mu\text{Ci}$  NDMA per flask in 50 mL.

<sup>b</sup>all flasks contained lake water with salts; glucose is given in grams per liter.

<sup>c</sup>changed three times the first week, twice a week for two weeks, once a week for seven weeks, and once the last two weeks.

<sup>d</sup>at the completion of the study, aliquots of the media were assayed for residual radioactivity.

<sup>e</sup>after 28 days.

<sup>f</sup>no data.

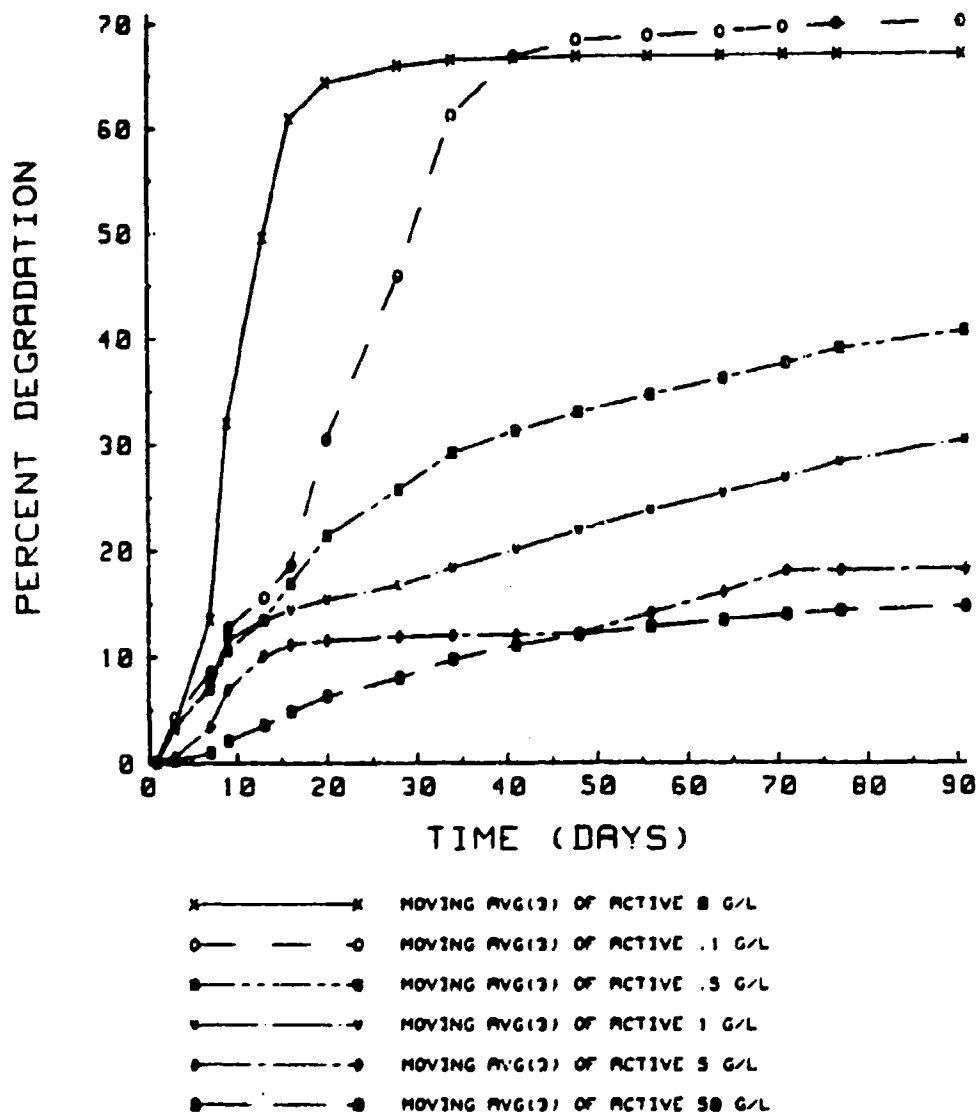


Figure 7. Mineralization of NDMA, 10 ppb, from lake water supplemented with glucose at various concentrations.

Table 8 and Figure 8 present results from a biodegradation study in soil, sand, and ashed soil; the concentration of NDMA was 40 ppb. Our previous report had presented results for a similar study but with NDMA at ppm concentrations.

TABLE 8. Total  $^{14}\text{C}$ -labeled Volatile Products Recovered in BT Study during 74 Days.<sup>a</sup>

| <u>Incubation conditions</u> | <u>Sterile (-)<br/>Active (+)</u> | <u>Base traps<sup>b</sup><br/>(% of total)</u> | <u>Acid traps<sup>b</sup><br/>(% of total)</u> |
|------------------------------|-----------------------------------|--|--|
| Soil <sup>c</sup>            | --                                | 1.8  | 0.9  |
| Sand <sup>d</sup>            | --                                | 1.5  | 1.4  |
| Ashed soil <sup>e</sup>      | --                                | 1.6  | 1.5  |
| Soil                         | +                                 | 65.5   | <sup>f</sup>                                   |
| Sand                         | +                                 | 74.9   | 0.8  |
| Ashed soil                   | +                                 | 0.1  | 1.3  |

<sup>a</sup>40 ppb NDMA and 0.081  $\mu\text{Ci}$  NDMA per flask. Soil, sand, and ashed soil were at pH 5.8, 5.5 and 8.0, respectively.

<sup>b</sup>changed twice a week for three weeks, once a week for five weeks, and once the last two weeks.

<sup>c</sup>25.0 g oven dry weight with 33.1 mL distilled water.

<sup>d</sup>25.0 g oven dry weight with 30.6 mL distilled water.

<sup>e</sup>25.0 g oven dry weight with 30.0 mL distilled water.

<sup>f</sup>no acid trap.

The results demonstrate that NDMA is rapidly mineralized from soil or sand at the 40 ppb concentration, while ashed soil did not provide suitable conditions for similar activity.

Table 9 and Figure 9 present results of a study that investigated the effect of moisture on the rates of mineralization of NDMA in soil. The results indicate that soil moisture has little effect on rates of mineralization of low concentration of NDMA in soils. Activity was suppressed only in the air dried soil, as would be expected since minimal moisture levels are required to prevent desiccation of microorganisms as well as for basic cell activities.

#### Cell Viability - NDMA Concentration.

Cell viabilities on both growth media, after exposure to different concentrations of NDMA were determined by colony counts after specific intervals. The data are presented in Table 10. Over the 24 hour period the total viable colony counts increased in both media. There was no indication of a toxic or inhibitory effect of NDMA at concentrations up to 1000 ppm on these cell populations.

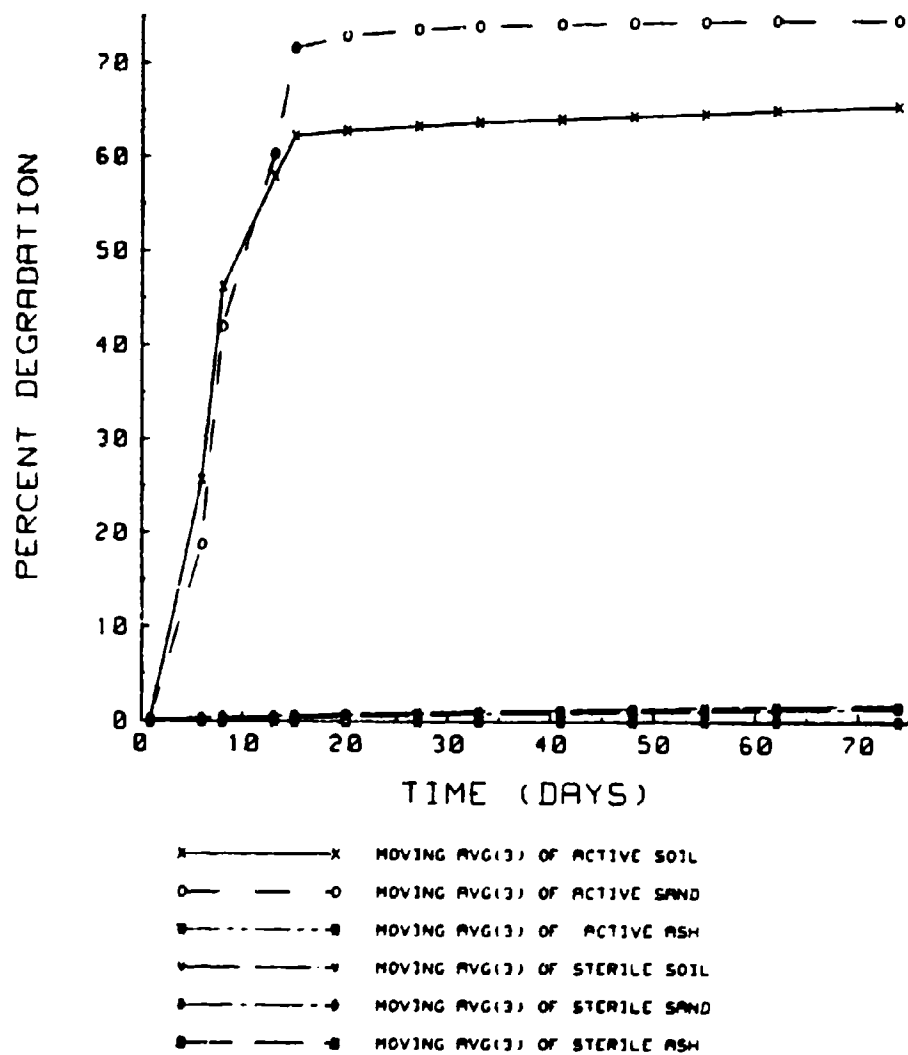


Figure 8. Mineralization of NDMA, 40 ppb, in soil, sand, and ashed soil.

TABLE 9. Total  $^{14}\text{C}$ -labeled Volatile Products Recovered in SW Study during 74 Days.<sup>a</sup>

| <u>Incubation conditions<sup>b</sup></u> | <u>Sterile (-)<br/>Active (+)</u> | <u>Base traps<sup>c</sup><br/>(% of total)</u> | <u>Acid traps<sup>b</sup><br/>(% of total)</u> |
|--|-----------------------------------|--|--|
| Air dried                                | +                                 | 1.1  | 0.3  |
| 25% moisture                             | +                                 | 50.3   | 0.3  |
| 50% moisture                             | +                                 | 47.6   | 0.4  |
| 75% moisture                             | +                                 | 52.6   | 0.6  |
| 100% moisture                            | +                                 | 57.0   | 0.4  |
| Air dried                                | -                                 | 1.4  | 1.3  |
| 25% moisture                             | -                                 | 0.4  | 1.5  |
| 50% moisture                             | -                                 | 0.8  | 1.8  |
| 75% moisture                             | -                                 | 0.2  | 1.3  |
| 100% moisture                            | -                                 | 0.4  | 0.8  |

<sup>a</sup>100 ppb NDMA and 0.081  $\mu\text{Ci}$  NDMA per flask. Soil pH was 5.8.

<sup>b</sup>in soil, percent of field capacity.

<sup>c</sup>changed twice a week for three weeks, once a week for five weeks and once the last two weeks.

TABLE 10. Effect of NDMA Concentration on Cell Viability.

A. Lake water + glucose agar

| <u>NDMA<br/>(ppm)</u> | <u>T = 0</u>      | <u>T = 1 hour</u> | <u>T = 24 hours</u> |
|-----------------------|-------------------|-------------------|---------------------|
| Control (no NDMA)     | 108.5 $\pm$ 29.0* | 34.0 $\pm$ 11.3   | 193.5 $\pm$ 61.5    |
| 1000                  | 94.5 $\pm$ 84.1   | 128.5 $\pm$ 26.2  | 373.5 $\pm$ 24.7    |
| 1                     | 153.0 $\pm$ 14.1  | 122.0 $\pm$ 56.6  | 215.5 $\pm$ 78.5    |
| 0.001                 | 139.5 $\pm$ 53.0  | 104.0 $\pm$ 24.0  | 255.5 $\pm$ 78.5    |

TABLE 10. Effect of NDMA Concentration on Cell Viability. (cont'd)

## B. Nutrient agar

| NDMA<br>(ppm)     | T = 0            | T = 1 hour       | T = 24 hours      |
|-------------------|------------------|------------------|-------------------|
| Control (no NDMA) | 185.5 $\pm$ 41.7 | 219.0 $\pm$ 43.8 | 311.0 $\pm$ 21.2  |
| 1000              | 268.5 $\pm$ 34.6 | 306.0 $\pm$ 34.4 | 549.5 $\pm$ 105.4 |
| 1                 | 279.5 $\pm$ 47.4 | 213.5 $\pm$ 53.0 | 371.5 $\pm$ 87.0  |
| 0.001             | 223.5 $\pm$ 62.9 | 195.0 $\pm$ 46.7 | 314.0 $\pm$ 0.0   |

\*average number of colony forming units per plate  $\pm$  1 Standard Deviation

Thin-layer Chromatography.

The following  $R_f$  values for hydrazine and hydrazine derivatives were obtained as a result of TLC analysis, hydrazine 0.33, 1, 1 dimethylhydrazine 0.76, and methylhydrazine 0.58. N-nitrosodimethylamine had an  $R_f$  of 0.80 but did not produce the blue color upon visualization. None of the unknowns from batch culture systems (chromatographed direct, acidified, or concentrated) showed evidence for hydrazine inter-mediate. Investigations for radioactively labeled spots on chromatograms corresponding to the standards also did not produce evidence for these hydrazines.

High Performance Liquid Chromatography.

Initial evidence for the identity of intermediates was obtained from analyses performed on the coupled HPLC-radioactivity detector system. The retention times of  $^{14}\text{C}$ -labeled standards are presented in Table 11.

TABLE 11. Retention Times of  $^{14}\text{C}$ -NDMA and other Standards that Represent Potential Biologically Produced Intermediates.

| Compounds<br>( $^{14}\text{C}$ -labeled) | Retention time (Min) |                        |
|--|----------------------|------------------------|
|  | UV detector (254 nm) | Radioactivity detector |
| N-nitrosodimethylamine                   | 2.7 - 3.3            | 3.9 - 4.7              |
| Methylamine                              | N.D.*                | 3.1 - 3.5              |
| Dimethylamine                            | N.D.                 | 5.5 - 5.7              |
| Formaldehyde                             | N.D.                 | 2.3 - 2.9              |

\*Not detectable



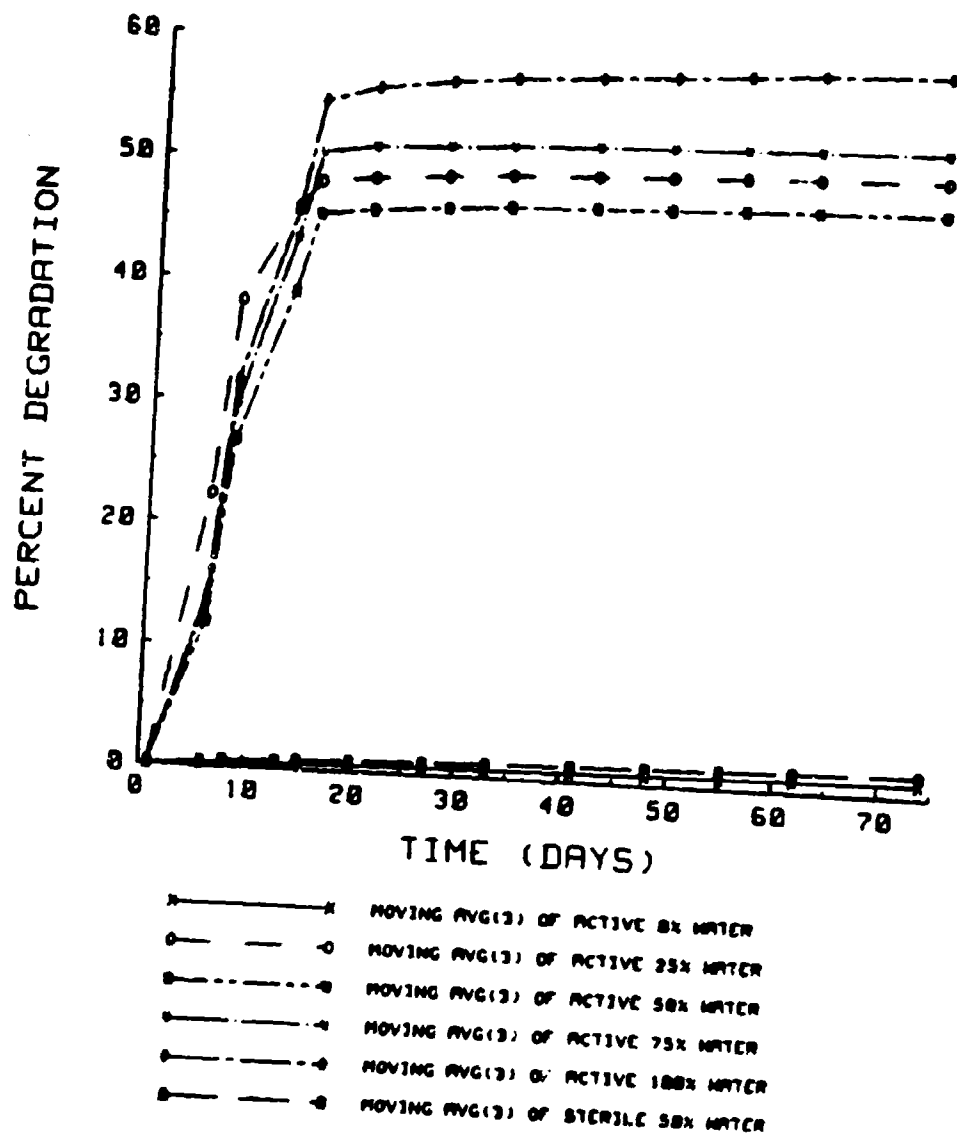


Figure 9. Mineralization of NDMA, 100 ppb, in soil with different moisture levels.

Results from analysis of samples from batch cultures with  $^{14}\text{C}$ -NDMA provided evidence for intermediates with retention times that correlated with the methylamine and formaldehyde standards. Both ultraviolet and radioactivity chromatograms illustrating these findings are presented in Figure 10.

#### Mass Spectrometry.

To confirm our results from the HPLC-radioactivity detector system, batch culture samples initiated with  $^{14}\text{C}$ -NDMA were analyzed on a SCIEX MS. The results of these analysis confirmed the presence of formaldehyde. In separate studies, both NDMA and  $^{14}\text{C}$ -NDMA (approximately 50 ppt to 100 ppt) in sterile distilled water were found to be stable over four weeks at room temperature.

#### Gas Chromatography - purge and trap.

The efforts to detect potential volatile intermediates from batch cultures by GC after concentration by purge and trap techniques proved unsuccessful. GC data for the standard compounds are presented in Table 12. None of the potential intermediates was detected other than NDMA, the starting substrate. Despite the purge and trap procedure the intermediates may not have been concentrated to detectable limits.

TABLE 12. Purge and Trap Results for NDMA and Potential NDMA Intermediates

| <u>Standard</u>             | <u>Concentration<br/>(ppm) in 50 mL</u> | <u>Retention<br/>time (min)</u> | <u>Responses<br/>(Area)</u> |
|-----------------------------|---|---------------------------------|-----------------------------|
| Methanol                    | 20                                      | 7.08                            | 38,150                      |
| Dimethylamine               | 5                                       | 5.66                            | 13,170                      |
| Methylamine                 | 50                                      | 4.41                            | 6,652                       |
| Formaldehyde                | 0.5                                     | 7.01                            | 12,630                      |
| N-nitrosodi-<br>methylamine | 0.4                                     | 8.96                            | 632,900                     |
| Chloroform                  | 20                                      | 11.40                           | 3,189,000                   |

#### Soil Binding.

In Figure 11, the results of the soil binding experiment with NDMA and humic acid are illustrated. The results indicate that there is little reaction between NDMA and humic acid under the experimental conditions investigated. If binding reactions were occurring there would be a decreasing concentration of unbound NDMA with time, but this was not the case.

#### Resting Cell and Cell Extract Studies.

It was not possible by a limited number of experiments to obtain resting cells or cell extracts active in mineralizing NDMA. This was the case for both adapted and unadapted whole cells and cell extracts.

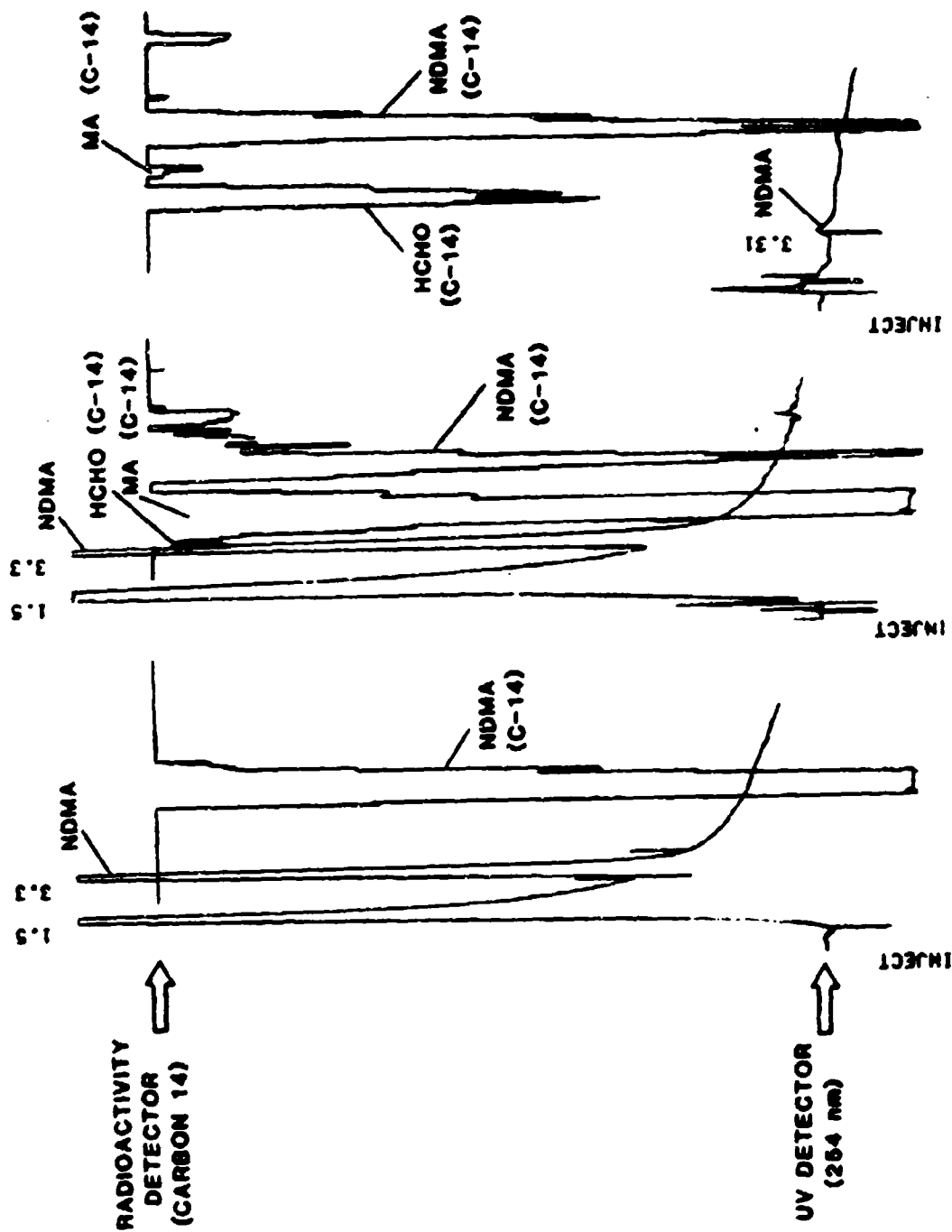


Figure 10. HPLC chromatograms illustrating the UV and radioactivity tracings of NDMA, formaldehyde (HCHO) and methylenamine (MA). The first run (left) is an NDMA standard, the next two tracings are representative of batch culture samples.

### Sorption on charcoal.

The sorption capacity of charcoal under the experimental conditions of this study is illustrated in Figure 12. The data for initial concentration of NDMA and the sorption of NDMA by the charcoal are transformed by log manipulations. Approximately 110 mg of NDMA is retained by 1.0 g of the charcoal, corresponding to 11 weight percent absorption.

### Kinetics

#### Mineralization - Measurement

Mineralization kinetics were evaluated using the  $^{14}\text{CO}_2$  recovery data from the batch aqueous and soil studies. Mineralization of  $^{14}\text{C}$ -NDMA is determined by the evolution of  $^{14}\text{CO}_2$  which is collected in alkaline traps. Measurements of radioactivity remaining in the incubation media were made at the termination of some of the experiments. Losses in radioactivity from the media were reflected by the recovery of  $^{14}\text{CO}_2$  in the base traps. Rates and extent of mineralization are used to quantify the biodegradation of NDMA under the different environmental conditions.

The recovery of  $^{14}\text{C}$  in base traps, as the measure of mineralization, assumes that most of this  $^{14}\text{C}$  is represented by  $^{14}\text{CO}_2$ . For these studies, this is a valid assumption. In general, only background quantities of  $^{14}\text{C}$  were recovered in the base traps in corresponding sterile controls; representing some volatility. Also some of the possible metabolites from NDMA (methylamine, dimethylamine, and hydrazines) would be collected in the acid traps while formaldehyde, methanol or methane would not be collected in either trap system. Therefore it is unlikely that significant portions of the  $^{14}\text{C}$  collected in the alkaline traps are due to compounds other than  $^{14}\text{CO}_2$ . As in any study on mineralization of organic compounds, a failure to demonstrate mineralization as reflected in  $\text{CO}_2$  production does not preclude biotransformation of the compound.

#### Mineralization - Aqueous

NDMA was incubated for 114 days at concentrations of 12.2 pg/mL to 15 ug/mL in lake water supplemented with salts, glucose, and nutrient broth. In general, the extent of mineralization increased with decreasing initial concentration of NDMA in the first two media (Table 13). In nutrient broth the extent of mineralization was reduced at all concentrations. At concentrations in the low ppb range and below there was a significant increase in extent of mineralization. This threshold level was found with both lake water with salts and lake water with salts and glucose.

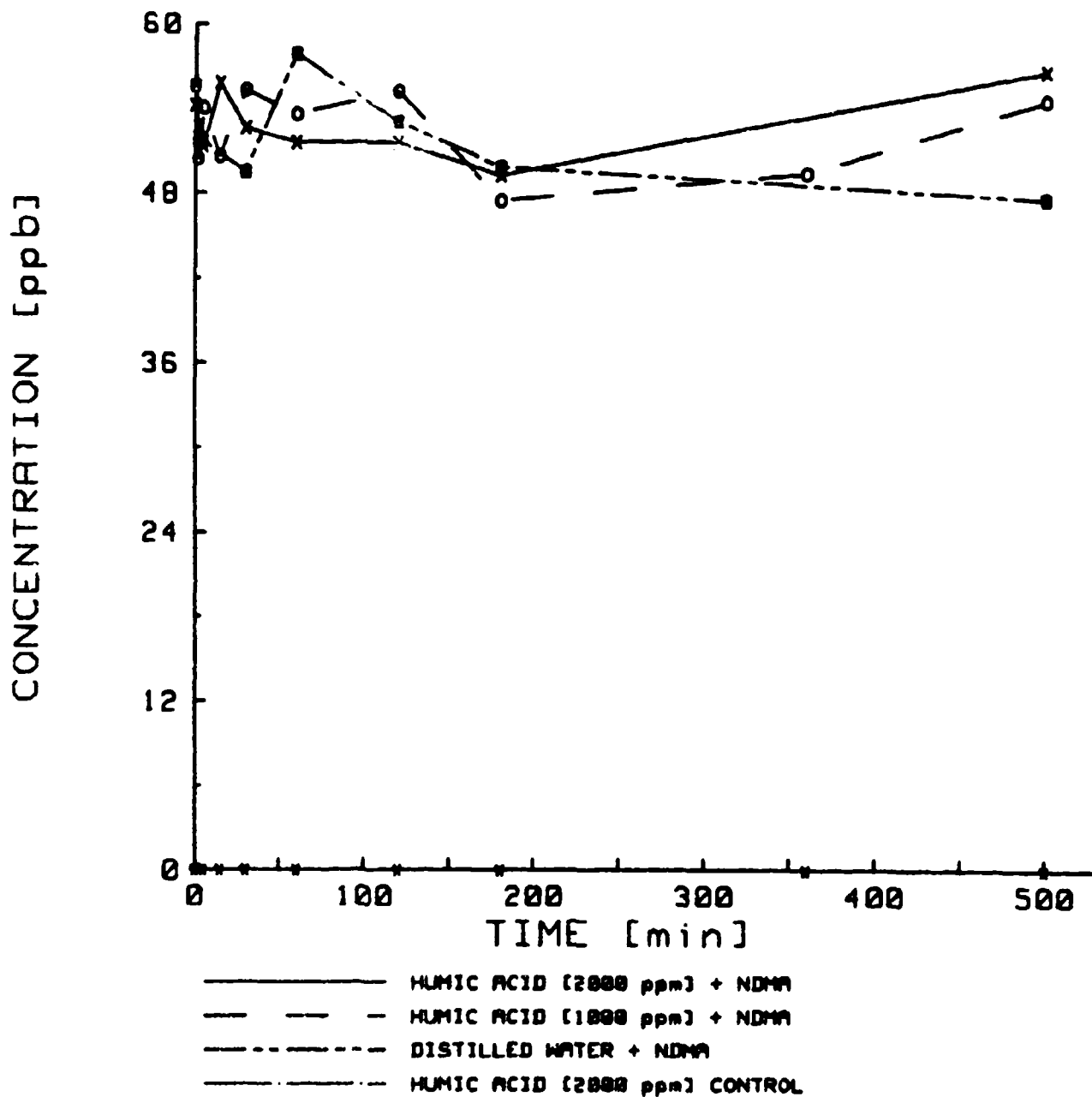


Figure 11. Soil binding experiment with NDMA, 50 ppb, and humic acid.

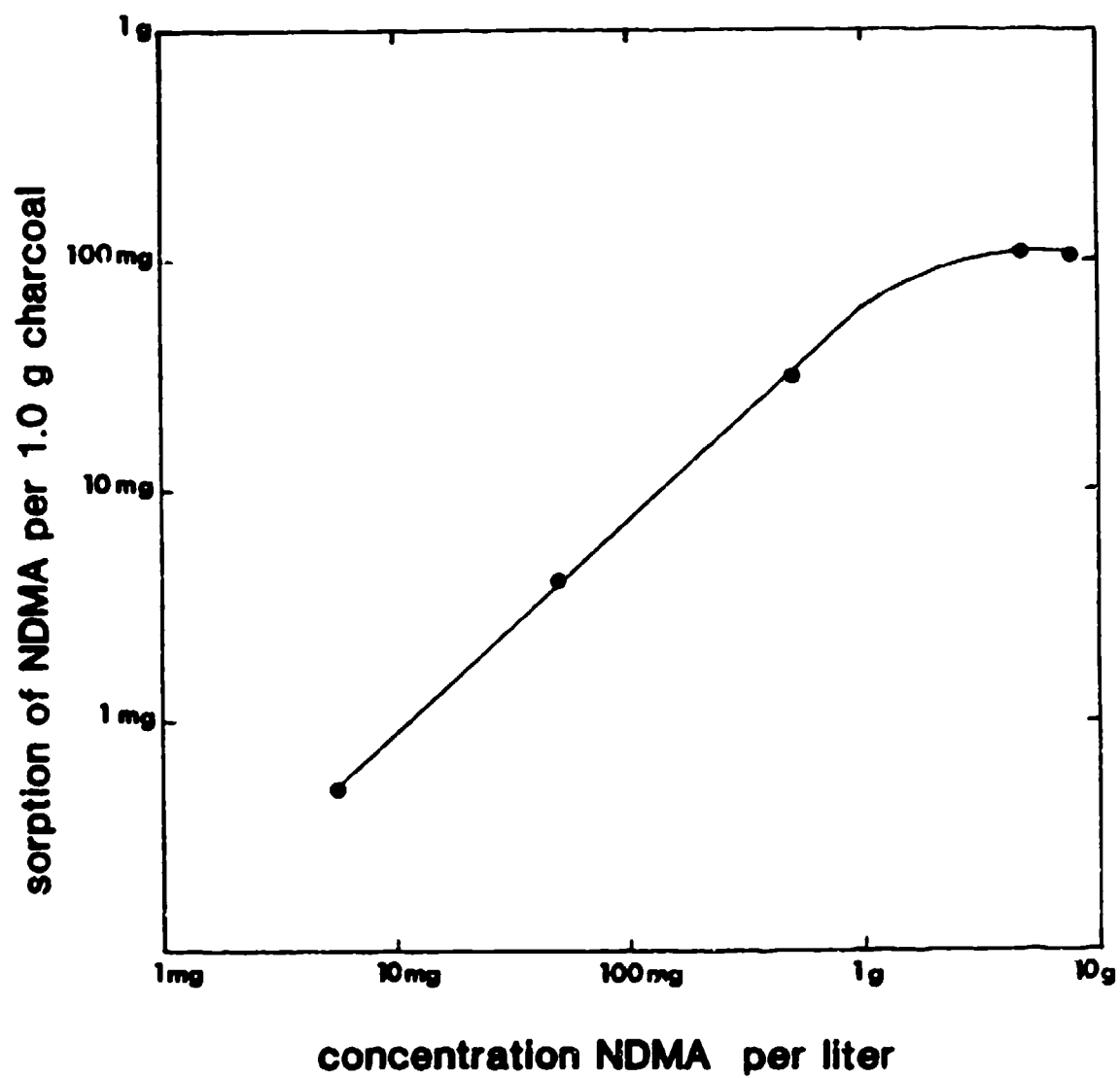


Figure 12. Sorption of NDMA by charcoal.

TABLE 13. Extent of Mineralization (percent) of NDMA during 114 Days.

| Initial<br>concentration<br>(ug/mL) | Lake water<br>(salts) | Lake water<br>(salts & glucose) | Lake water<br>(nutrient broth) |
|-------------------------------------|-----------------------|---------------------------------|--------------------------------|
| $1.50 \times 10^1$                  | 16.4                  | 18.4                            | — <sup>b</sup>                 |
| $1.50 \times 10^0$                  | 21.4                  | 24.3                            | —                              |
| $1.50 \times 10^{-1}$               | 32.0                  | 31.4                            | —                              |
| $1.50 \times 10^{-2}$               | 69.0                  | 48.0                            | 27.1                           |
| $1.51 \times 10^{-3}$               | 76.1                  | 67.2                            | 19.1                           |
| $1.62 \times 10^{-4}$               | 78.6                  | 55.4                            | 35.2                           |
| $2.72 \times 10^{-5}$               | 70.5                  | 58.8                            | 17.4                           |
| $1.22 \times 10^{-5}$               | 65.5                  | 90.7                            | 23.0                           |

<sup>a</sup>  $\pm$  1 standard deviation for bracket grouping.

<sup>b</sup> no data

No lag phase was evident in the lake water with salts and salts with glucose media. Initial rates of mineralization (weight  $\times$  mL<sup>-1</sup>  $\times$  day<sup>-1</sup>) were calculated for the linear portion of the <sup>14</sup>CO<sub>2</sub> recovery curves (13 days) (Table 14). Rates of mineralization were greatly reduced after about four weeks of incubation and there was only low recovery of <sup>14</sup>CO<sub>2</sub> for the remainder of the experiment. Linear regressions were calculated for the initial mineralization rates and correlation coefficients were generally between 0.95 and 0.99. These initial mineralization rates then plotted (log plots) against the initial concentration of NDMA (Figure 13).

Within the range of concentrations tested there was a linear relationship between initial rate of mineralization and initial concentration of NDMA; or the rate of degradation was directly proportional to concentration. This contrasts with the results from measurements of the extent of mineralization where the total percent degraded was not directly proportional to concentration but inversely related. The slopes of the regression lines in Figure 13. are 0.907, 0.894 and 1.038 for lake water with salts, lake water with salts and glucose and nutrient broth, respectively.

Rate constants (initial rate of mineralization/initial concentration NDMA) were calculated for each incubation condition (Table 15). The rate constants increased significantly as the initial concentration of NDMA decreased. There is over a 3.7-fold increase between the highest concentration grouping and the lowest concentration grouping (Table 15) in lake water with salts, and with salts and glucose. The rate constants are higher for the lake water with salts without glucose than with the glucose at all concentrations. However, in nutrient broth, the rate constants were lowest for all incubations. These findings would indicate inhibition of degradation at all NDMA concentrations with addition of supplemental carbon.

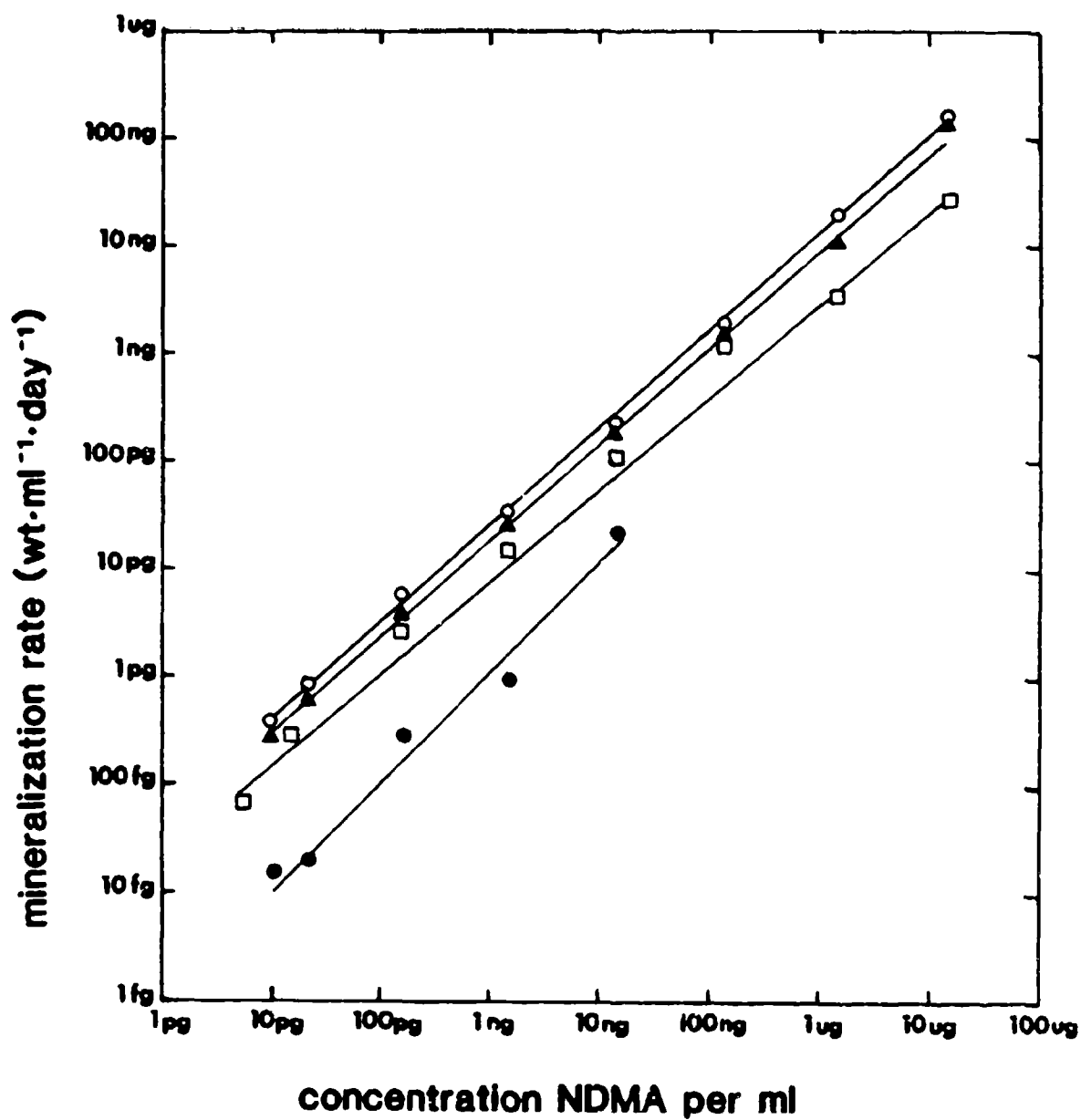


Figure 13. Initial rates of mineralization over a range of concentrations of NDMA in aqueous batch culture. Open circles represent lake water with salts, closed circles represent nutrient broth, open squares represent lake water, and closed triangles represent lake water with salts and glucose.



TABLE 14. Calculations of Initial Mineralization Rates.

Mineralization Rate ( $\mu\text{g} \times \text{mL}^{-1} \times \text{day}^{-1}$ )

| Initial<br>Concentration<br>( $\mu\text{g}/\text{mL}$ ) | Lake water<br>(salts) | Lake water<br>(salts & glucose) | Lake water<br>(nutrient broth) |
|---|-----------------------|---------------------------------|--------------------------------|
| $1.50 \times 10^1$                                      | $1.70 \times 10^{-1}$ | $1.10 \times 10^{-1}$           | *                              |
| $1.50 \times 10^0$                                      | $2.00 \times 10^{-2}$ | $1.00 \times 10^{-2}$           | ---                            |
| $1.50 \times 10^{-1}$                                   | $1.93 \times 10^{-3}$ | $1.71 \times 10^{-3}$           | ---                            |
| $1.50 \times 10^{-2}$                                   | $2.38 \times 10^{-4}$ | $1.81 \times 10^{-4}$           | $2.93 \times 10^{-5}$          |
| $1.51 \times 10^{-3}$                                   | $3.46 \times 10^{-5}$ | $2.16 \times 10^{-5}$           | $8.38 \times 10^{-7}$          |
| $1.62 \times 10^{-4}$                                   | $6.55 \times 10^{-6}$ | $3.92 \times 10^{-6}$           | $3.65 \times 10^{-7}$          |
| $2.72 \times 10^{-5}$                                   | $9.59 \times 10^{-7}$ | $6.25 \times 10^{-7}$           | $1.77 \times 10^{-8}$          |
| $1.22 \times 10^{-6}$                                   | $4.02 \times 10^{-7}$ | $3.88 \times 10^{-7}$           | $1.66 \times 10^{-8}$          |

\* no data

TABLE 15. Calculation of Rate Constants

RATE CONSTANTS

 $(\mu\text{g} \times \text{mL}^{-1} \times \text{day}^{-1} / \mu\text{g} \times \text{mL}^{-1})$ 

| Initial<br>Concentration<br>( $\mu\text{g}/\text{mL}$ ) | Lake water<br>(salts) | Lake water<br>(salts & glucose) | Lake water<br>(nutrient broth) |
|---|-----------------------|---------------------------------|--------------------------------|
| $1.50 \times 10^1$                                      | 0.0113                | 0.0073                          | 0.0070 $\pm$                   |
| $1.50 \times 10^0$                                      | 0.0133                | 0.0067                          | 0.0004 <sup>a</sup>            |
| $1.50 \times 10^{-1}$                                   | 0.0129                | 0.0114                          | ---                            |
| $1.50 \times 10^{-2}$                                   | 0.0159                | 0.0121                          | 0.0126 $\pm$                   |
| $1.50 \times 10^{-3}$                                   | 0.0229                | 0.0143                          | 0.0015                         |
| $1.62 \times 10^{-4}$                                   | 0.0404                | 0.0242                          | 0.0263 $\pm$                   |
| $2.72 \times 10^{-5}$                                   | 0.0353                | 0.0230                          | 0.0047                         |
| $1.22 \times 10^{-6}$                                   | 0.0330                | 0.0318                          | 0.0014                         |

<sup>a</sup> average  $\pm$  standard deviation<sup>b</sup> no data

Another experiment to evaluate the role of NDMA concentration on mineralization kinetics was conducted over 89 days in lake water with concentrations of NDMA from 6.78  $\mu\text{g/mL}$  to 30  $\mu\text{g/mL}$ . As before, the extent of mineralization increased with decreasing initial concentration of NDMA (Table 16). Overall, the extent of mineralization was below that found in the previous study (lake water with salts and salts and glucose, Table 13). Initial rates of mineralization were calculated over the first twenty days of incubation and then plotted against the initial concentration of NDMA, as before (Figure 13). The slope of this regression line is 0.857.

Table 16. Mineralization of NDMA in Lake Water.

| Initial concentration<br>( $\mu\text{g/mL}$ ) | Extent of mineralization<br>(% during 89 days) | Initial rate of mineralization<br>(20 days) | Rate constants        |
|---|--|---|-----------------------|
| $3.00 \times 10^1$                            | 2.8  | $3.00 \times 10^{-2}$                       | $1.00 \times 10^{-3}$ |
| $3.00 \times 10^0$                            | 3.5  | $3.59 \times 10^{-3}$                       | $1.19 \times 10^{-3}$ |
| $3.00 \times 10^1$                            | 24.8   | $2.13 \times 10^{-3}$                       | $7.10 \times 10^{-3}$ |
| $3.00 \times 10^{-2}$                         | 17.1   | $1.12 \times 10^{-4}$                       | $3.73 \times 10^{-3}$ |
| $3.00 \times 10^{-3}$                         | 28.7   | $1.57 \times 10^{-5}$                       | $5.22 \times 10^{-3}$ |
| $3.07 \times 10^{-4}$                         | 39.4   | $3.20 \times 10^{-6}$                       | $1.04 \times 10^{-2}$ |
| $3.68 \times 10^{-5}$                         | 41.2   | $2.96 \times 10^{-7}$                       | $8.05 \times 10^{-3}$ |
| $6.78 \times 10^{-6}$                         | 38.7   | $6.15 \times 10^{-8}$                       | $9.07 \times 10^{-3}$ |

All correlation coefficients for the linear regressions fit for initial rates of mineralization were above 0.95. As with the previous results over the range of concentration tested, there was a linear relationship between the rate of mineralization and initial concentration of NDMA. Rate constants increased as the initial concentration of NDMA decreased, with an 8.4-fold increase from the lowest grouping of concentrations to the highest.

In other aqueous batch studies, conducted with 12.12  $\text{ng/mL}$  NDMA, supplemental carbon was further evaluated for its role in degradation kinetics. In Table 17 the effect of glucose additions in lake water on mineralization of NDMA is shown. As the initial concentration of glucose increased, the extent of mineralization decreased. The initial rate of mineralization was highest for the unsupplemented lake water; 4.7-fold less for the 0.1 to 5.0  $\mu\text{g/mL}$  grouping; and 15.2-fold less for the incubation with the highest glucose concentration. Rate constants followed a similar pattern. In this experiment all indicators of mineralization showed a decreasing measure of activity as the concentration of glucose was increased.

Table 17. Effect of Glucose on Degradation Kinetics of NDMA in Lake Water.

| Glucose<br>( $\mu\text{g/mL}$ ) | Extent of<br>mineralization<br>(%) (90 days) | Initial rate<br>of mineralization<br>(13 days) | Rate constant         |  |
|---------------------------------|--|--|-----------------------|--|
| 0                               | 67.1   | $6.71 \times 10^{-4}$                          | $5.54 \times 10^{-2}$ |  |
| 0.1                             | 70.2   | $1.52 \times 10^{-4}$                          | $1.25 \times 10^{-2}$ |  |
| 0.5                             | 40.7   | $1.33 \times 10^{-4}$                          | $1.10 \times 10^{-2}$ | $1.18 \times 10^{-2} \pm$<br>$1.16 \times 10^{-3}$ |
| 1.0                             | 30.4   | $1.58 \times 10^{-4}$                          | $1.30 \times 10^{-2}$ |  |
| 5.0                             | 18.2   | $1.28 \times 10^{-4}$                          | $1.06 \times 10^{-2}$ |  |
| 50.0                            | 14.7   | $4.42 \times 10^{-5}$                          | $3.64 \times 10^{-3}$ |  |

In a separate study with 12.12 ng/mL NDMA, lake water was supplemented with salts, salts and glucose, or nutrient broth. All experiments were replicated with and without a chemical reducing agent, 0.025% sodium sulfide (Table 18).

TABLE 18. Effect of Nutrient Supplements on Degradation Kinetics of NDMA in Lake Water.

| Lake water<br>supplement | Extent of<br>mineralization<br>(%), (90 days) | Initial rate<br>of mineralization<br>(12 days) | Rate constant         |
|--------------------------|---|--|-----------------------|
| Salts                    | 64.1  | $9.07 \times 10^{-4}$                          | $7.48 \times 10^{-2}$ |
| Salts & Glucose          | 10.6  | $9.29 \times 10^{-5}$                          | $7.67 \times 10^{-3}$ |
| Nutrient broth           | 13.6  | $8.57 \times 10^{-6}$                          | $7.07 \times 10^{-4}$ |
| Salts & red.*            | 60.1  | $1.04 \times 10^{-3}$                          | $8.58 \times 10^{-2}$ |
| Salts & glucose & red.   | 13.6  | $1.69 \times 10^{-4}$                          | $1.39 \times 10^{-2}$ |
| Nutrient broth & red.    | 10.8  | $7.93 \times 10^{-6}$                          | $6.54 \times 10^{-4}$ |

\* reducing agent

Supplemental carbon inhibited activity in all cases as reflected by the different measurements of mineralization activity. The addition of the reducing agent had no effect on this pattern.

#### Mineralization - Soil

The effect of concentration of NDMA on mineralization in soil was studied in batch incubations run for 98 days at concentrations ranging from 10 ng/g soil to 10 mg/g soil (Table 19).

TABLE 19. Mineralization of NDMA in Soil.

| <u>Initial concentration<br/>(<math>\mu\text{g/g}</math> soil)</u> | <u>Extent of mineralization<br/>(%) (98 days)</u> | <u>Initial rate of mineralization<br/>(7 days)</u> | <u>Rate constant</u> |
|--|---|--|----------------------|
| 10,000.00  | 28.6  | $1.64 \times 10^2$                                 | 0.0164               |
| 1,000.00   | 24.4  | $1.37 \times 10^1$                                 | 0.0137               |
| 100.00   | 29.0  | $1.36 \times 10^0$                                 | 0.0136               |
| 1.00   | 55.3  | $2.00 \times 10^{-2}$                              | 0.0200               |
| 0.01   | 78.4  | $7.02 \times 10^{-4}$                              | 0.0702               |

No lag phase was noted before the onset of the initial activity. Initial rates of mineralization ( $\mu\text{g} \times \text{g}^{-1} \times \text{day}^{-1}$ ) were calculated over the first seven days of incubation. Correlation coefficients for these linear regressions were above 0.95. As with the aqueous studies on concentration of NDMA the initial rates of mineralization were plotted against the initial concentration (Figure 14). Over the range of concentrations tested, there was a linear relationship between initial rate of mineralization and NDMA concentration, again indicating degradation rates are related to concentration. Also, as was shown in the aqueous studies, the extent of mineralization and the rate constants were highest for the lowest initial concentration (1  $\mu\text{g/g}$  soil and 0.01  $\mu\text{g/g}$  soil). The slope of the regression line in Figure 14. is 0.900, in close agreement with the findings in the aqueous studies (Figure 13).

The effect of soil organic matter on degradation of NDMA was evaluated (Table 20). At the highest concentration of organic matter the rate and extent of mineralization was reduced.

Table 20. Effect of Organic Matter on the Degradation of NDMA (100.2  $\mu\text{g/g}$  soil) in Soil.

| <u>Organic matter<br/>(%)</u> | <u>Extent of mineralization<br/>(% over 119 days)</u> | <u>Initial Rate of mineralization<br/>(30 days)</u> | <u>Rate constant</u> |
|-------------------------------|---|---|----------------------|
| 35.0                          | 40.0  | $9.60 \times 10^{-4}$                               | 0.0096               |
| 10.5                          | 36.0  | $1.42 \times 10^{-3}$                               | 0.0142               |
| 5.3                           | 54.3  | $1.41 \times 10^{-3}$                               | 0.0141               |
| 2.6                           | 60.9  | $1.01 \times 10^{-3}$                               | 0.0101               |

The influence of moisture levels on the degradation of NDMA in soil was also evaluated (Table 21). Almost all activity was inhibited in the dry soil, the extent of mineralization was similar at all other moisture levels, while initial rates were higher for the 25% level compared with the three highest moisture levels.

TABLE 21. Effect of Moisture on Degradation of NDMA (102.2 µg/g soil) in Soil.

| Moisture<br>(% of field<br>capacity) | Extent of<br>mineralization<br>(% over 74 days) | Initial rate<br>of mineralization<br>(8 days) | Rate constant         |
|--------------------------------------|---|---|-----------------------|
| 0                                    | 1.1   | $3.27 \times 10^{-5}$                         | $3.27 \times 10^{-4}$ |
| 25                                   | 50.3  | $1.00 \times 10^{-2}$                         | $9.80 \times 10^{-2}$ |
| 50                                   | 47.6  | $3.39 \times 10^{-3}$                         | $3.32 \times 10^{-2}$ |
| 75                                   | 52.6  | $3.79 \times 10^{-3}$                         | $3.71 \times 10^{-2}$ |
| 100                                  | 57.0  | $3.81 \times 10^{-3}$                         | $3.73 \times 10^{-2}$ |

In a separate experiment supplemental carbon or salts (salts, glucose, cellulose) was added to soil containing 1 µg/g soil NDMA. The degradation kinetics showed little difference in rates as reflected by initial mineralization rates and rate constants.

### DISCUSSION

#### Mineralization Kinetics.

Many factors may influence the kinetics of mineralization of chemicals including substrate concentration, microbial population (substrate utilizing microorganisms), hydrogen ion concentration, inorganic and organic nutrients, the presence of inhibitory compounds or synergistic interactions, temperature, levels of oxygen and other gases, competition with binding and polymerization reactions, bacterial predation, attachment sites for microorganisms, and retention time in the system. The complex interactions between these factors as well as the experimental difficulties in trying to measure all of these potential factors would preclude any attempt to mathematically model such systems in a comprehensive and highly accurate manner. To predict the fate and half-lives of chemicals. A truly useful model should incorporate enough of these factors to allow its use in the accurate prediction of the mineralization kinetics over as wide a range of environmental situations as possible.

There has been considerable discussion regarding the type of kinetic model which most accurately predicts mineralization rates for organic chemicals by natural populations.<sup>3,4</sup> Most often bacterial populations and substrate concentration are chosen as the two factors for the development of predictive models. Even low concentrations of organic chemicals can be quantified by sensitive instrumentation as well as by the use of radioactive tracers. However, the accurate quantitation of natural microbial populations actively involved in the mineralization process is much more difficult.

Kinetics provides a clearer more quantitative picture of the interactions between chemicals and environmental factors, as reflected in reaction rates or mineralization rates. Some important factors involved in this approach are:

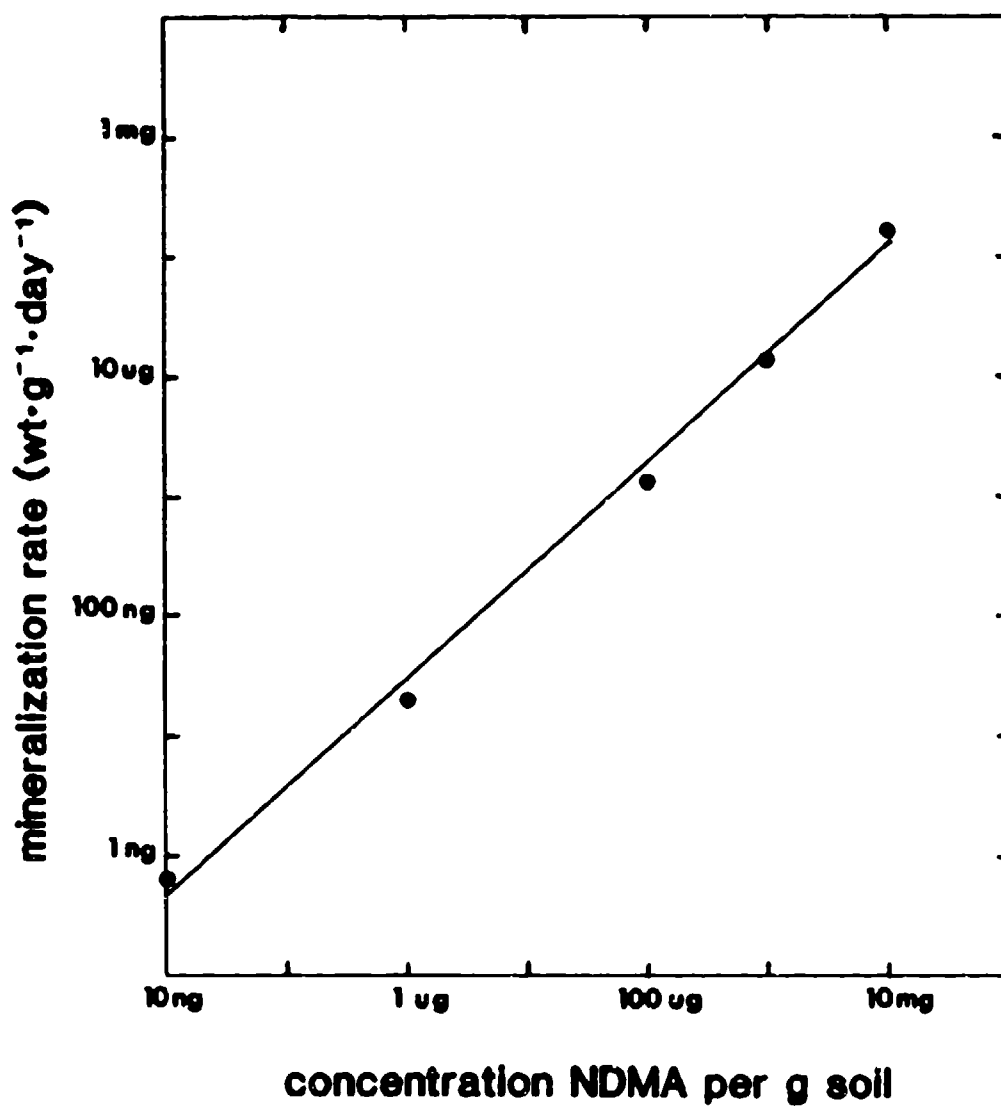


Figure 14. Initial rate of mineralization over a range of concentrations of NDMA in soil batch culture.

(1) Time of incubation.

Reaction rates must be measured during the initial period of incubation where rates are maximum and the mineralization curve appears to be linear. These rates must be measured after a lag phase (if one occurs) and before a decline in activity. These considerations are especially important in batch culture systems where inhibitory effects can appear rapidly.

With NDMA, the time frame for initial rates was in days, indicating slow metabolism when compared to much shorter time frames for more readily metabolized compounds. In general, no significant lag phase was seen in most of the batch studies with NDMA.

(2) Organisms.

Studies involving degradation kinetics may include measurements of microbial populations as important factors in rate equations. However, if microbial populations are included in these calculations, it is not appropriate to use total cell populations as a measure of degradation activity. Only cell counts for organisms which possess the ability to degrade the chemical of interest should be considered in determining the role of biomass in degradation kinetics. In this regard, Bartholomew and Pfander<sup>5</sup> reported that most xenobiotics are present at very low concentrations in the environment, and the aquatic populations will be inactive the majority of time. They claimed, therefore, that measurements of population numbers may not be reflective of the metabolic activities carried out by a relatively small portion of the community. The authors also found that biodegradation rates did not correlate well with the general characteristics of the microbial community, such as temperature, microbial community size, and amino acid turnover rate.

In our attempts to isolate NDMA-degrading organisms it was found to be very difficult to achieve and maintain significant colony growth on NDMA-containing media without supplemental carbon. With this added carbon it was not certain that growth was solely on the NDMA or on the supplemental carbon as well. We have therefore excluded consideration of NDMA-degrading population measurements by assuming equivalent initial populations in all cases. All experiments were initiated in a similar manner with respect to the inoculum and therefore the initial populations should be similar.

(3) Concentration.

A number of reports have discussed the role of concentration on rates of degradation.<sup>6-10</sup> The importance of carrying out laboratory studies at "realistic" concentrations of a chemical, in light of the levels expected or found in natural systems, has been recognized. It can be argued that studies carried out over a broad range of concentrations are necessary in order to fully assess all possible levels of environmental contamination and make extrapolations to natural systems. Wang et. al, 1984,<sup>9</sup> have recently demonstrated that isopropyl N-phenylcarbamate is mineralized at low concentration (400 ppt) in lake water but only cometabolized and not mineralized at higher concentration (1 ppm).

For Michaelis-Menton kinetics, to achieve saturation and thus calculate  $V_{max}$ , high concentrations of the compound under study may have to be used. With NDMA, we studied an eightfold order of magnitude range (ppt to ppm) of concentrations, however, at the highest concentration saturation kinetics was not attained, thus preventing calculation of  $V_{max}$ . It is clear that for the range of concentrations tested, the rate of mineralization of NDMA from aqueous and soil systems was directly proportional to the initial concentration of NDMA, as would be predicted for a fit with first-order Michaelis-Menton kinetics.

In terms of kinetic theory, Michaelis-Menton kinetics as originally applied to enzyme catalyzed reactions are often used to describe degradation kinetics by whole organisms. A linear response between substrate concentration (the compound of interest) and reaction rate (mineralization as in  $^{14}CO_2$  recovery) is indicative of a fit with Michaelis-Menton kinetics. With NDMA such a fit was found. The mineralization rates are first order (directly dependent on substrate (NDMA) concentration) because we have eliminated the microbial biomass or NDMA-degrading populations measurements as a variable for reasons discussed above.

The effect of low concentration on rates and extent of mineralization of aromatic and aliphatic organic compounds has recently been studied<sup>6-10</sup>. Mineralization of organic compounds at low concentrations has been shown to "fit" a variety of kinetic models. It is unclear which kinetic model is most applicable in predicting mineralization rates at low concentrations. In any case, however, it is important to realize that if Michaelis-Menton kinetic theory holds, as it appears to in the case of NDMA, differences in concentrations must be considered when extrapolations are made from laboratory to natural concentrations. Rates of degradation may be dramatically different unless comparable concentrations are evaluated.

Measurements of mineralization rates are calculated from initial rates of  $^{14}CO_2$  release. The extent of mineralization, however, is long term and is influenced by a number of factors other than just initial substrate concentration. These other factors theoretically should not come into play during the initial reactions, but may do so, especially in batch studies. These factors may include a buildup of inhibitory products, secondary changes in the media such as a change in pH, or effects involving cell walls or transport mechanisms. Any or all of these factors, or even other unidentified effects may explain why initial rates of mineralization are highest at higher initial concentrations of NDMA, yet extent of mineralization and the rate constants fall in the reverse order. Limited attempts were made to remove the effects of the cell wall using disrupted cells but the results were unclear.

In soils it is often postulated that different kinetics may apply due to the fact that nutrients and the compound under study are not uniformly distributed in the soil. In the case with NDMA a linear relationship was found between initial mineralization rate and concentration as had been found in aqueous solution. Apparently the high solubility of NDMA overcomes the problems of diffusibility throughout water films covering soil particles.



A number of authors have discussed the role of ecologically distinct natural populations of microorganisms (eutrophic, mesotrophic, oligotrophic) in these degradation processes. Subba-Rao et al., 1982, have suggested that these populations respond differently when exposed to different concentrations of a compound.<sup>9</sup> Rates of mineralization may increase at low substrate concentrations because of a shift in the compound-utilizing population. In support of this theory, Subba-Rao et al., 1982, found rate constants for phenol, aniline, 2,4-dinitroaniline, and p-nitrophenol decreased at higher initial substrate concentrations. Rubin and Alexander, 1983 found that trace levels of certain compounds in eutrophic environments may not be metabolized.<sup>8</sup> Wang et. al., 1984, postulated a role for eutrophic and oligotrophic populations in lake water in cometabolism vs mineralization of isopropyl N-phenylcarbamate in lake water. These authors further speculate that even a single population may cometabolize or mineralize an organic substrate depending on its concentration.<sup>9</sup> In our studies, the effect of NDMA concentration on cell viability indicated no such shift between oligotrophic and eutrophic populations.

As an alternative to the above theory, much research on the effect of low substrate concentration on rates of mineralization has shown that threshold levels may be reached below which degradation decreases or ceases. This effect is usually attributed to the fact that insufficient energy is provided by the substrate at these low concentrations. Minimum maintenance energy requirements of the cell populations can not be met under these conditions leading to a decline in cell population or in cell activity. Studies which support of this theory indicate that substrate concentration may be a significant factor in limiting rates of biodegradation in natural systems. This was not the case with NDMA.

#### Continuous and Batch Culture Systems.

There was no significant degradation of NDMA in continuous flow systems under aerobic or anaerobic conditions at ppm or ppb concentrations. Experiments with a laboratory scale column comprising a trickling filter with a charcoal bed, indicate that this treatment configuration has potential for degradation of NDMA. NDMA was mineralized by this column and almost completely removed under continuous flow conditions from the feed line which contained either 50 ppm or 100 ppm of the nitrosamine. Apparently the charcoal bed and column configuration provided the proper environment to promote the degradation of NDMA. This may be due to the enhancement of NDMA-degrading microorganisms, a change in the NDMA structure upon sorption to the charcoal, which results in an enhanced susceptibility to catabolic enzymes, or simply sorption to the charcoal possibly providing for a longer retention time in combination with the column conditions. When the same type of charcoal was added to batch cultures there was virtually a complete cessation of mineralization of NDMA; which may indicate the significant role the column environment played in the activity found with the trickling filter.

The batch studies provided information on the kinetics of mineralization of NDMA under a variety of environmental conditions. The initial concentration of NDMA was critical in determination of rates of mineralization. Two phenomena were demonstrated experimentally. First, total mineralization of NDMA from the batch studies was inversely related to initial concentration of NDMA. At lower ppb and ppt concentrations, high total mineralization of NDMA was achieved, while at higher concentrations only limited total mineralization was found. Second, when initial rates of activity (mineralization) were measured, the data demonstrate a direct linear relationship between initial concentration of NDMA and reaction rate (mineralization). As the initial concentration of NDMA increased, the initial rate of mineralization increased. The seeming discrepancy between these two measures of activity was discussed previously and a number of possible explanations were offered (build-up of inhibitory products in batch culture; secondary changes in media, cell wall, or cell transport effects, the activities of two or more ecologically distinct populations of microorganisms with optimal activities at different concentrations of NDMA, or other unidentified effects). Similar patterns of mineralization were found in both aqueous and soil systems.

In general, supplemental carbon was found to be inhibitory to mineralization of NDMA. NDMA was not inhibitory to soil microorganisms at concentrations up to 1000 ppm.

#### Biotransformation Pathway.

Grilli and Prodi, 1975, identified the products of metabolism of NDMA in rats.<sup>11</sup> Oxidative dealkylation with rat liver microsomes, led to the formation of formaldehyde, formic acid, methylamine, and N-methylhydrazine. With the soluble cell enzyme extract reductive reactions led to the formation of N-methylhydroxylamine and N,N-dimethylhydrazine (Figure 15).

We found no evidence for a reductive pathway in bacterial cells. No hydrazine or hydrazine derivatives were detected. Evidence was found for formaldehyde and methylamine as intermediates and thus a scheme shown in figure 16 is proposed for microbial degradation of NDMA.

#### Treatment Options and Recommendations

The results of this work indicate limited applicability of a biological treatment mode for the removal of NDMA from wastewater, with one qualification. In general, at environmental concentrations in the upper ppb and ppm range, only limited degradation of NDMA is achieved. This is the case in continuous culture as well as in batch studies. The one qualification is the trickling filter system with which excellent results were obtained under the laboratory conditions evaluated. A recommendation for future work would be to scale up the column to pilot scale to evaluate performance with different NDMA loading rates, different charcoal, and different feed rates, and different influent media. There may be potential for the use of this granular activated charcoal column for the biological treatment of NDMA-laden wastewaters.

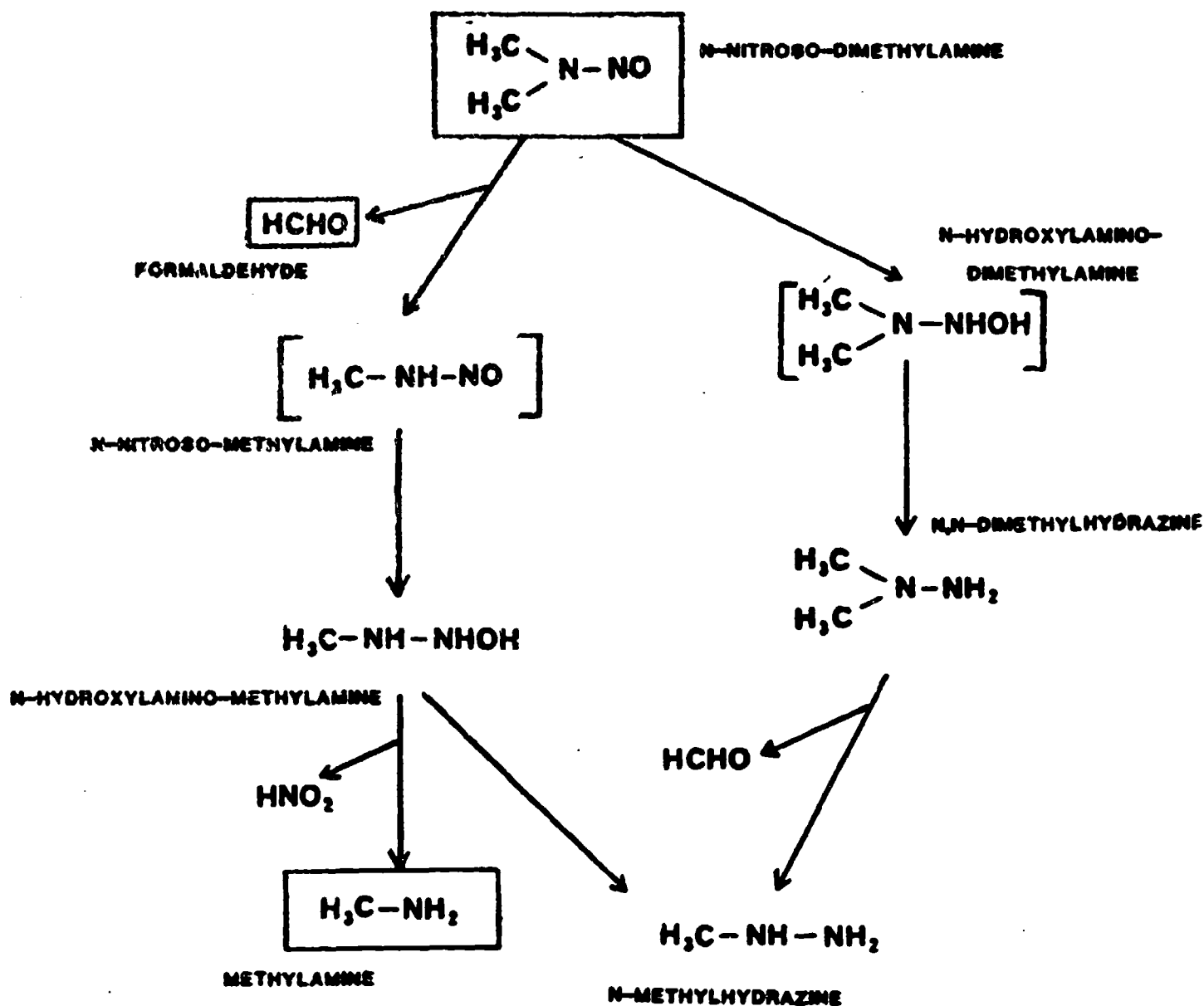


Figure 15. Summary biodegradation scheme for potential intermediates formed from NDMA. Boxed compounds were identified during microbial degradation in this study. Bracketed compounds and dotted lines, are postulated intermediates and pathways, respectively. Much of the reductive pathway to hydrazines and the oxidative pathway was identified by Grilli and Prodi, 1975, with mammalian systems.



## CONCLUSIONS

N-Nitrosodimethylamine (NDMA) is biodegradable under certain conditions. In batch studies with aqueous and soil incubations, initial rates of mineralization increased with increasing concentration of NDMA (ppt up to ppm). However, the total percent or extent of mineralization decreased with increasing initial concentration of NDMA. Supplemental carbon was inhibitory to NDMA degrading activity. NDMA was not inhibitory to soil microorganisms at concentrations up to 1000 mg/liter. With one exception, continuous culture systems with a range of NDMA concentrations and conditions failed to demonstrate an ability to degrade a significant percentage of NDMA.

The only approach involving biological treatment that appears to show significant NDMA-degrading capability was a continuous flow column containing granular activated charcoal (simulated trickling filter). This column demonstrated an ability to mineralize NDMA at feed concentrations of 50 ppm and 100 ppm and may warrant further investigation to evaluate its potential use in an actual mode for treatment of NDMA-laden wastewaters. Formaldehyde and methylamine were identified as transitory intermediates formed during the biodegradation of NDMA. A biotransformation pathway is proposed for the microbial degradation of NDMA based on these observations.

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